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Supercritical carbon dioxide inactivation technology for food product preservation☆

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ABSTRACT

Supercritical carbon dioxide ($scCO_2$) is increasingly recognized as a non-thermal food preservation technology capable of ensuring microbial safety while preserving nutritional and sensory quality. Operating at moderate temperatures (<50 °C), $scCO_2$ offers an environmentally friendly alternative to thermal pasteurization, leveraging its unique physicochemical properties to penetrate cells, induce acidification, and disrupt microbial structures. This review provides a comprehensive overview of the antimicrobial mechanisms of $scCO_2$, including effects on membranes, enzymes, and intracellular pH, as well as its limitations in low-moisture systems and against spores, which require higher temperature, water, or synergistic co-treatments. The inactivation of viruses and fungal spores is also discussed, highlighting the potential of $scCO_2$ in combination with additives such as hydrogen peroxide or natural antimicrobials. Attention is given to sublethal injury and viable-but-non-culturable (VBNC) states, which pose risks for microbial recovery and underscore the need for robust detection and storage strategies. The review explores hurdle technology approaches, integrating $scCO_2$ with pulsed electric fields, high-power ultrasound, high-pressure processing, and food-grade additives to enhance lethality while maintaining product integrity. Current applications remain limited to pilot- or small-scale operations, with promising results in juices, dairy, and ready-to-eat foods. However, industrial adoption faces persistent challenges such as high equipment costs, regulatory uncertainties, and limited scalability. Future efforts should prioritize standardization of process parameters, validation of continuous systems, and regulatory harmonization. Overall, $scCO_2$ emerges as a versatile, sustainable, and gentle alternative for microbial inactivation, well-suited to meet consumer demand for safe, minimally processed foods.

1. Introduction

Ensuring food safety while preserving sensory and nutritional quality remains a major challenge for the food industry. In this context, supercritical carbon dioxide ($scCO_2$) has emerged over the past few decades as a promising non-thermal technology for microbial inactivation. $scCO_2$ can operate at moderate temperatures (<50 °C) than conventional thermal pasteurization, thereby minimizing detrimental effects on food texture and bioactive compounds. $scCO_2$ refers to carbon dioxide above its critical point (31.1 °C and 7.38 MPa), where its exhibits both the diffusivity of a gas and the solvent power of a liquid. This unique dual behavior enables CO_2 to penetrate biological membranes, disrupt cellular functions, and inactivate microorganisms while preserving the sensory and nutritional quality of food products (Buszewski et al., 2022; Veiga et al., 2024; Yu et al., 2020).

Supercritical carbon dioxide is also recognized as an environmentally sustainable technology for food processing. The CO_2 used can be recovered from industrial waste streams, or, more sustainably, derived from biogenic processes such as fermentation, thereby promoting carbon recycling and reducing net greenhouse gas emissions (Alonso-Moreno and García-Yuste, 2016).

$scCO_2$ leaves negligible chemical residues in treated products, aligning with consumer demand for clean-label and minimally processed foods (Roobab et al., 2021). The $scCO_2$ inactivation process typically involves three mains phases: pressurization, treatment (holding time), and depressurization (Fig. 1). Initially, the system is pressurized to achieve supercritical conditions, during which CO_2 transitions into its supercritical state. During the treatment phase, the product is

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held under these conditions for a defined time, allowing $scCO_2$ to inactivate microorganisms. Finally, controlled depressurization returns the CO_2 to its gaseous phase, which can be recaptured and recycled, there enhancing the process's sustainability. $scCO_2$ can be applied in batch or continuous systems, with batch processes manly used in research and pilot-scale studies, while continuous systems are preferred for industrial scale-up applications. Most available data come from batch or semi-batch configurations; however, results can be extrapolated to continuous systems by applying mathematical models based on equivalent resident (holding) time (Paniagua-Martínez et al., 2018).

Process parameters, including temperature, pressure, resident time, and pressurization/depressurization rates, must be carefully optimized according to the food matrix and target microorganisms. Indeed, inactivation efficiency strongly depends on both microbial type and food matrix properties, with substantial variability across heterogeneous systems, such as porous solids versus viscous liquids, which complicates protocol standardization (Garcia-Gonzalez et al., 2009; Veiga et al., 2024). Industrial adoption of $scCO_2$ is still hindered by technical and economic constraints, which limit its scalability and widespread implementation.

This review critically examines the state of the art of $scCO_2$ technology in its transition from laboratory concept to industrial application, with emphasis on antimicrobial mechanisms (including sublethal injury) and synergistic hurdle combinations. It also addresses scalability challenges, considering current and future trends as well as economic and environmental trade-offs.

2. State of the art

2.1. Inactivation mechanisms of $scCO_2$

2.1.1. Vegetative microbial cell

$scCO_2$ exerts its antimicrobial activity through a combination of physicochemical mechanisms involving both acidification and structural damage. The inactivation process depends on the ability of CO_2 to reach its supercritical state, which enables it to penetrate microbial cells and disrupt vital cellular functions. In the presence of free water, CO_2 dissolves and forms carbonic acid (H_2CO_3); however, at food-relevant pH values (typically below 6), the dissociation of H_2CO_3 into protons is limited. Therefore, the observed intracellular acidification is primarily attributable to the diffusion of CO_2 across the cell membrane and the consequent collapse of the proton motive force, leading to equilibration of intracellular and extracellular pH (Garcia-Gonzalez et al., 2007; Li et al., 2023; Tamburini et al., 2014). This disturbance in proton homeostasis impairs pH-sensitive enzymatic systems (e.g., ATPases, dehydrogenases) and contributes to the metabolic arrest observed during $scCO_2$ treatment. The cytoplasmic environment becomes increasingly acidic (pH <5), promoting the precipitation of divalent ions (Ca^{2+}, Mg^{2+}), disruption of osmoregulation, and inhibition of redox metabolism (Garcia-Gonzalez et al., 2007). Membrane solubilization is further enhanced by the direct interaction between $scCO_2$ and phospholipid bilayers, particularly in Gram-negative bacteria. Cellular damage is not limited to pH-mediated effects: rapid pressurization and depressurization during $scCO_2$ treatment can cause mechanical rupture, especially in microorganisms with thin or less rigid cell walls. During depressurization, intracellular leakage of proteins, nucleic acids, and ions results in irreversible cell damage (Li et al., 2023; Tamburini et al., 2014).

Electron microscopy provides visual confirmation of cell damage, such as membrane collapse and surface deformation, but it does not elucidate the underlying mechanism of inactivation. The lethal effect of $scCO_2$ has instead been associated with biochemical and physical alteration, including intracellular acidification, inhibition of key enzymes (e.g., ATPase, dehydrogenases), leakage of ions and metabolites, and disruption of membrane phospholipids (Chen et al., 2017; Garcia-Gonzalez et al., 2007, 2009; Tamburini et al., 2014).

2.1.2. Bacterial spore

Although $scCO_2$ alone can inactivate vegetative microorganisms, its efficacy against bacterial spores at low temperatures remains limited. Spore inactivation by $scCO_2$ involves multiple synergistic factors: heat-induced germination enhances permeability, the high gas-phase diffusivity breaches the multilayered spore barriers, and carbonic acid formation contributes to the denaturation of core proteins and enzymes. All this effects can be modulated by pressure, exposure time, and by combining $scCO_2$ with other treatments (Hart et al., 2022). Spilimbergo et al. (2002) demonstrated that a 24 h treatment at 70 bar and 75 °C achieved more than a 7 log CFU reduction of *Bacillus subtilis* spores, whereas at 36 °C under the same pressure only a 0.5 log CFU decrease was observed (Spilimbergo et al., 2002). This strong temperature dependence likely reflects heat-triggered germination: once germination occurs, the cortex and inner membrane become more permeable to CO_2,

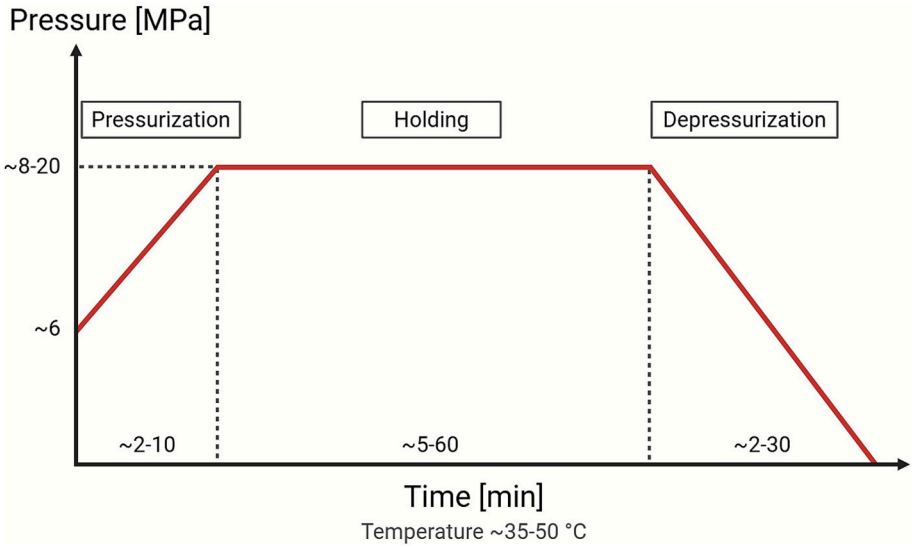

Fig. 1. Schematic representation of the supercritical carbon dioxide ($scCO_2$) batch treatment profile, showing the three main process phases: pressurization, holding, and depressurization. Pressure is increased from ~6 MPa to ~8–20 MPa over ~2–10 min, maintained for ~5–60 min, and then released to atmospheric levels within ~2–30 min. The process is typically conducted at temperatures of ~35–50 °C.

and the increased $scCO_2$ diffusivity at higher temperatures further accelerates penetration and inactivation (Spilimbergo et al., 2002). However, germination induced during $scCO_2$ processing should not be considered a preservation strategy. Spore populations are intrinsically heterogeneous and contain subpopulations that remain dormant (superdormant spores) even under favorable germination conditions. Germination can therefore only sensitize spores, not ensure full inactivation, and reliance on this mechanism alone cannot achieve regulatory lethality targets in food matrices.

Ballestra and Cuq (1998) likewise reported that high-pressure CO_2 may trigger partial germination in aqueous suspensions, leading to dipicolinic acid (DPA) release and membrane softening, which increase susceptibility to subsequent $scCO_2$-induced damage (Ballestra and Cuq, 1998). In practical terms, effective spore inactivation at low temperatures requires combining $scCO_2$ with an additional hurdle, such as moderate heat, oxidizing agents, or other stresses, to achieve pasteurization-equivalent reductions.

Nevertheless, $scCO_2$ can also directly inactivate dormant spores under sufficiently harsh conditions. Hart et al. (2022) reported complete inactivation of *B. subtilis* spores at 82–91 °C and 6.5–25 MPa in under 2 h, indicating a non-germinative mechanism driven by mechanical disruption and intracellular acidification (Hart et al., 2022).

2.1.3. Fungal spore

The inactivation of fungal spores by $scCO_2$ involves a combination of chemical and physical effects that results in structural damage and cell death. Initially, CO_2 dissolves in the thin aqueous layer surrounding the spore, forming carbonic acid. This acidification lowers the local pH, leading to swelling and weakening the spore coat and cortex (Noman et al., 2018).

Compared to bacterial spores, fungal spores such as *Aspergillus* conidia lack a complex multilayered structure. Specifically, they lack a dehydrated core enriched in DPA or a cortex protected by multiple protein coats. As a result, their resistance to $scCO_2$ is generally lower, and the inactivation mechanism relies more heavily on membrane destabilization and oxidative damage, likely caused by the reactive oxygen species generated during treatment (Hart et al., 2022; Zhang et al., 2007).

Under high pressure, CO_2 penetrates deeper into the spore core, facilitated by enhanced membrane permeability. Scanning electron microscopy images clearly show fungal spores appearing distorted, collapsed, or cracked after $scCO_2$ treatment, indicating severe structural damage (Noman et al., 2018; Park et al., 2015).

Temperature further enhances this process by increasing membrane fluidity and promoting CO_2 diffusion. Moderate heating (approximately 55–75 °C) can also initiate germination, temporarily increasing spore susceptibility to damage (Noman et al., 2018).

Inside the spore, $scCO_2$ extracts essential lipid components from the cell membrane, compromising its integrity, and denatures structural and metabolic proteins, such as enzymes involved in energy production and stress response, through acidification and direct interaction with CO_2.

Spores with a spherical shape, such as those of *Aspergillus* species, tend to collapse evenly across their surface under supercritical CO_2, whereas elongated spores, such as those of *Penicillium* species, concentrate mechanical stress at their narrower tips and therefore rupture first at those points (Noman et al., 2018). The combined effects of membrane disruption, oxidative stress, and enzyme inactivation lead to irreversible loss of metabolic activity and spore viability (Noman et al., 2016).

In addition to conidia, certain filamentous fungi, such as *Byssochlamys fulva* and Aspergillus *fischeri,* form heat-resistant ascospores, which pose a considerable challenge in food preservation. Their exceptional resistance derives from a thick multilayered wall and a highly dehydrated core, both of which confer remarkable thermal stability. Experimental data on their response to CO_2-based inactivation treatments are limited. Ballestra and Cuq (1998) demonstrated that exposing *B. fulva* ascospores to pressurized CO_2 at 5 MPa during heating at 80 °C enhanced the inactivation rate, producing approximately an 80 % reduction in viable spores, whereas heat alone had no effect under identical conditions. This enhancement disappeared at 85 °C, suggesting that the synergistic contribution of CO_2 is limited to a narrow sublethal range. To date, no study has demonstrated the complete inactivation of heat-resistant fungal ascospores by $scCO_2$. These observations indicate that this spore type remains largely unaffected under mild $scCO_2$ conditions and continues to represent a key limitation for CO_2-based sterilization processes.

2.1.4. Virus

The inactivation of viruses by $scCO_2$ is a complex process that often requires the addition of co-solvents to achieve complete inactivation. Several studies have demonstrated that the virucidal efficacy of $scCO_2$ is significantly enhanced by co-solvent or adjuvants such as ethanol, water, hydrogen peroxide, or peracetic acid, which facilitate deeper CO_2 penetration, promote acidification, and generate reactive species capable of inducing irreversible oxidative damage to viral proteins and lipids (Martino et al., 2022; Perrut, 2012).

Depending on the virus type and process parameters, typical conditions for enveloped virus inactivation range from 35 to 40 °C, 80–100 bar, and 15–60 min in the presence of co-solvents, achieving reductions of $\geq$6 log. Non-enveloped viruses generally require harsher conditions, such as 40–60 °C, 150–300 bar, and 1–2 h with oxidizing additives, whereas neat $scCO_2$ without adjuvants often demands longer exposure times ($\geq$ 2 h) or higher pressures ($>$ 150 bar) to achieve comparable levels of inactivation (Perrut, 2012).

These findings suggest that the use of neat $scCO_2$ alone may be insufficient to achieve robust viral inactivation, particularly for non-enveloped viruses. However, recent work by Ruiz et al. (2025) reported effective inactivation of SARS-CoV-2 using $scCO_2$ without the need for chemical additives, under conditions of 100 bar, 35 °C and 5 min, indicating that viral susceptibility to $scCO_2$ may vary substantially depending on both the virus type and processing conditions (Ruiz et al., 2025). The underlying mechanism involves the rapid diffusion of $scCO_2$ into the viral capsid or envelope, facilitated by its low viscosity and high diffusivity, which leads to swelling and mechanical destabilization of the viral structure (Martino et al., 2022). In moist environments, $scCO_2$ dissolves to form carbonic acid, inducing local pH reductions that alter protein conformation and function (Perrut, 2012). For enveloped viruses, such as coronaviruses, lipid extraction from the viral membrane further compromises its integrity and exposes internal components (Bennet et al., 2021). Although non-enveloped viruses, such as Hepatitis A virus, are generally more resistant due to the absence of a lipid envelope, $scCO_2$ can still induce structural damage by destabilizing capsid proteins, thereby compromising the capsid's ability to protect the viral genome (Perrut, 2012).

Most of these observations are derived from model systems, with electron microscopy consistently showing $scCO_2$-treated viruses that lose their typical morphology, often with partially or completely ruptured structures (Bennet et al., 2021; Martino et al., 2022), whereas studies involving actual food matrices remain scarce.

2.2. Sublethal injury and recovery

Although $scCO_2$ treatments are widely used to achieve complete microbial inactivation, their efficacy is often limited by the occurrence of sublethal injury. In this physiological state, microbial cells lose their ability to grow on selective media but remain viable and capable of recovery. Such cells may exhibit membrane damage, disrupted ion gradients, impaired metabolism, or enzyme inhibition, yet maintain sufficient structural integrity to restore normal function under favorable post-treatment conditions. Sublethal injury is not unique to CO_2-based treatments, as similar effects have been observed following pulsed electric field processing (García et al., 2005), thermal pasteurization (Cebrián et al., 2017), and high-intensity ultrasound (Bermudez-Aguirre

and Niemira, 2022), indicating that this physiological state is a general phenomenon across various preservation technologies. In $scCO_2$ treatment sublethal injury has been widely reported in vegetative cells such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Enterococcus faecalis*, and *Salmonella Typhimurium* (Bi et al., 2015; Yuk and Geveke, 2011). A representative example of sublethal injury following $scCO_2$ treatment was reported by Yuk and Geveke (2011), who investigated the inactivation of *Lactiplantibacillus plantarum* in apple cider using a continuous-flow system operating at 7.6 MPa, with a residence time of 20 min and outlet temperatures ranging from 34 °C to 42 °C. Interestingly, although the extent of injury increased with temperature, a marked decrease in the proportion of sublethally injured cells was observed at 42 °C, indicating a transition toward complete inactivation. Sublethal damage was assessed by plating treated samples on both non-selective and selective media, thereby allowing differentiation between sublethally injured and fully inactivated cells. Furthermore, shelf-life testing indicated that storage at 4 °C effectively suppressed the regrowth of surviving L. *plantarum* over 28 days, underscoring the importance of combining non-thermal processing with proper cold-chain management to ensure microbial stability (Yuk and Geveke, 2011).

The use of selective media after lethality treatments may lead to an underestimation of viable counts, as such conditions suppress the recovery of sublethally injured cells. For reliable lethality assessment, enumeration on non-selective recovery media supplemented with repair-promoting additives is recommended to detect all potentially viable cells (Ceylan et al., 2021; Schottroff et al., 2018). The use of selective media in earlier studies should be interpreted with caution, as it may have led to and overestimation of the inactivation efficacy of $scCO_2$.

The presence of sublethally injured cells also raises concerns regarding the viable but non-culturable (VBNC) state, a survival condition in which bacteria remain alive and metabolically active but cannot grow on standard culture media. These two physiological states may occur simultaneously, or one may progress into the other over time (Yang et al., 2025). While sublethal injury often involves damage to the cell membrane or proteins, VBNC cells exhibit more profound alterations, such as reduced metabolism and altered gene expression.

In contrast, Zhao et al. (2016) proposed that CO_2-induced stress may trigger a VBNC-like state, but their study lacked replication and assessed viability only through propidium-iodide permeability, which reflects membrane integrity instead of metabolic activity. These findings should therefore be interpreted with caution. More robust work by Vilhena et al. (2019) demonstrated that cold-stressed *E. coli* K-12 cells could regain culturability when exposed to pyruvate under suitable resuscitation conditions, emphasizing that confirming a VBNC states requires evidence of metabolic reactivation rather than solely dye-based staining. Detecting these forms requires more advanced analytical methods than simple plate counts. Sublethally injured cells can be revealed using high-osmolarity recovery media, whereas VBNC cells are better identified using flow cytometry, viability PCR, or digital PCR combined with dyes that indicate membrane damage (Pan, 2020). Despite recent progress, it remains unclear whether and how sublethal injury may evolve into the VBNC state under $scCO_2$ treatment. Moreover, most evidence comes from controlled laboratory conditions, and little is known about the fate of such cells in actual food matrices. Future studies should focus on developing more sensitive detection tools, clarifying the mechanisms of recovery, and assessing the potential risks posed by sublethally injured or VBNC cells in $scCO_2$-processed foods.

2.3. Hurdle technology for food pasteurization

$ScCO_2$, together with other non-thermal technologies such as pulsed electric fields (PEF), high-power ultrasound (HPU), high-pressure processing (HPP), and the use of antimicrobial additives, represents a promising alternative to thermal treatments for microbial inactivation. While each of these technologies offers unique advantages in terms of energy efficiency, environmental impact, and preservation of food quality, none of them alone is universally effective across all food matrices and microbial targets. For this reason, the hurdle technology concept, defined as the strategic combination of multiple preservation methods, has emerged as a powerful approach to enhance overall process efficacy. In the specific case of $scCO_2$, combining it with other non-thermal techniques can compensate for its limitations, particularly in solid or heterogeneous food system, where gas diffusion is slower and uneven. These limitations would otherwise require more severe processing conditions (e.g., higher temperatures, longer exposure), which could negatively affect overall product quality. By using milder conditions across complementary technologies, hurdle approaches can achieve greater microbial inactivation while preserving texture, nutrients, and sensory characteristics (Arbal et al., 2024; Bigi et al., 2023). While synergistic effects are frequently observed in hurdle technology applications, growing evidence suggests that these outcomes result from the concurrent disruption of multiple cellular targets, including membrane permeabilization, intracellular acidification, oxidative stress, inhibition of stress-response system, and damage to enzymes, nucleic acids, and osmotic regulation. The application of multiple stressors at sublethal intensities can disrupt microbial homeostasis, induce metabolic exhaustion, and inhibit the synthesis of stress-response proteins (Bigi et al., 2023). Nevertheless, the precise interactions, sequence and relative importance of these effects remain poorly understood, and further research is needed to elucidate the molecular basis of the synergistic antimicrobial responses. The main hurdle combinations explored in conjunction with $scCO_2$ are summarized in Table 1.

2.3.1. Additives + $scCO_2$

Under dry or low-moisture conditions, the antimicrobial activity of $scCO_2$ decreases markedly, as the formation of carbonic acid requires the presence of free water. In such environments, both vegetative bacterial cells and spores exhibit high resistance, and effective inactivation is achieved only when $scCO_2$ is combined with additional stressors such as oxidizing agents and/or moderate heat. Warambourg et al. (2023) demonstrated that dry, additive-free $scCO_2$ (35–60 °C, $\leq$ 200 bar) produced less than a 1 log reduction of *Bacillus subtilis* spores inoculated on dry glass or polymer surfaces. Conversely, the addition of 200 ppm of H_2O_2 achieved more than an 8 log reduction at 110 bar and 40 °C after 20 min. When the pressure was reduced to 60 bar (subcritical CO_2) at the same H_2O_2 concentration, only $\approx$ 0.5 log reduction was obtained, confirming that synergy with antioxidants occurs only in the supercritical phase (Warambourg et al., 2023). These results indicate that neither dry nor subcritical CO_2 is effective in the absence of reactive additives. Water availability and additive chemistry, rather than the supercritical state alone, govern the lethality of CO_2-based processes in low-moisture systems.

Hydrogen peroxide (H_2O_2) is the most extensively studied additive, known to enhance spore inactivation via oxidative damage and acidification. In the same study, $scCO_2$ combined with 2.3 % H_2O_2 at 40 °C and 110 bar achieved >6-log reduction of *Bacillus atrophaeus* spores on polymeric medical devices within 60 min (Warambourg et al., 2023). The main advantage of H_2O_2 lies in its decomposition into water and oxygen, leaving negligible residues. In the food industry, its use is permitted as a technological adjuvant in both the US and in the EU, provided that no detectable residues remain.

This synergistic behavior supports the broader view that moisture or reactive additives are essential for microbial inactivation, whereas dry, additive-free $scCO_2$ alone is largely ineffective. In food applications, other agents such as nisin, a GRAS-approved antimicrobial peptide, have also been shown to act synergistically with $scCO_2$. Rao et al. (2016) reported that treating *Bacillus subtilis* spores with $scCO_2$ (20 MPa, 84–86 °C) and 0.02 % nisin simultaneously achieved a 4.1-log reduction, outperforming either treatment alone or in sequential application. Mechanistically, $scCO_2$ compromises the protective coat and cortex layers, facilitating nisin penetration into the inner membrane where it binds lipid II and form disruptive pores. This was confirmed by increased

Table 1
Hurdle combinations enhancing microbial inactivation by $scCO_2$.

Hurdle component	Primary stress induced by hurdle	Overlap with $scCO_2$ mechanism	Expected synergistic outcome	Reference
Hydrogen peroxide (H_2O_2)	Oxidative stress through Reactive oxygen species (ROS) generation; oxidation of proteins, lipids, and DNA	$scCO_2$ drives intracellular acidification and membrane perturbation; H_2O_2 adds ROS-mediated oxidative injury	Directionally synergistic: high spore reductions reported with oxidants + $scCO_2$. Effect is H_2O_2-dose-, species-, and matrix-dependent.	(Warambourg et al., 2023)
Nisin (bacteriocin)	Lipid II binding leading to pore formation; active mainly on Gram-positive vegetative cells or germinating spores	CO_2 increases membrane permeability and acid stress, facilitating nisin action on vegetative cells and spore outgrowth inhibition	Enhanced killing of Gram-positive vegetative cells and inhibition of spore outgrowth; spore lethality increases if germination is induced (matrix- and species-dependent).	(Rao et al., 2016)
Essential oils / terpenes (thymol, limonene, linalool)	Membrane solubilization, lipid disruption, and permeability changes	$scCO_2$ can solubilize hydrophobic terpenes into membranes	Additive membrane permeabilization and improved CO_2/terpene partitioning enhance inactivation; effect magnitude is matrix- and aw-dependent.	(Chen et al., 2022; Santi et al., 2025)
High-Power Ultrasound (HPU)	Acoustic cavitation, shear, localized heating	Cavitation enhances CO_2 dissolution and mass transfer, accelerating acidification kinetics	Supported for liquids; significantly shorter treatment times and comparable microbial reductions vs $scCO_2$ alone.	(Castillo-Zamudio et al., 2021; Morbiato et al., 2019)
Pulsed Electric Fields (PEF)	Electroporation of cell membranes; transient pore formation	Both target membrane integrity and intracellular pH; PEF facilitates CO_2 entry and acidification	Electroporation facilitates CO_2 entry and acidification; higher inactivation rates enabling lower P/T or shorter times (organism- and matrix-dependent).	(Pataro et al., 2010; Spilimbergo et al., 2003)
High-Pressure Processing (HPP)	Protein denaturation, enzyme inactivation, and membrane compression	CO_2 acidification sensitizes cells to pressure-induced damage	For vegetative cells, dissolved CO_2 allows lethality at 150–300 MPa vs 300–600 MPa without CO_2; spores generally require higher T/P. The effect is Matrix-dependent.	(Perez-Won et al., 2020; Rotabakk and Rode, 2023)
Moderate heat ($\leq$70 °C)	Increased membrane fluidity; may induce partial spore activation (sensitization)	Warmer $scCO_2$ improves diffusivity and acidification kinetics	Enhanced CO_2 penetration and intracellular acid stress accelerate inactivation of spores and vegetative cells	(Arbal et al., 2024; Hart et al., 2022; Spilimbergo et al., 2002)
pH adjustment / natural acidifiers	Pre-acidification reduces cellular buffering and weakens cytoplasmic pH control	Reinforces CO_2-driven intracellular acidification and membrane permeabilization	Allows lower operating pressures and shorter exposure times while maintaining lethality; kinetics stabilized across matrices.	(Garcia-Gonzalez et al., 2007; Veiga et al., 2024)
Sequential multi-hurdle (PEF + $scCO_2$ + HPP)	Electroporation of membranes (PEF), CO_2-mediated intracellular acidification, and pressure-induced protein/enzyme denaturation	Integrates multiple $scCO_2$-compatible mechanisms	Demonstrated synergistic enzyme inactivation and extended shelf-life in coho salmon with minimal quality loss	(Perez-Won et al., 2020) Pérez-Won et al., 2021

dipicolinic acid release and enhanced membrane permeability measured by flow cytometry (Rao et al., 2016).

Natural antimicrobials such as essential oils and terpenes - including thymol, linalool, and limonene -have also shown strong synergy with $scCO_2$, particularly in low-moisture foods. Chen et al. (2022) demonstrated that pre-soaking raw almonds in thyme oil followed by $scCO_2$ treatment (10 MPa, 70 °C, 30 min) resulted in a 5.16-log reduction of *E. coli* K12, compared with <3-log for $scCO_2$ alone (Chen et al., 2022). Similarly, Santi et al. (2025) confirmed the enhanced efficacy of limonene and linalool in reducing microbial loads on fresh meat when combined with $scCO_2$ (Santi et al., 2025).

To mitigate sensory issues associated with volatile residues, Casas et al. (2016) proposed an additional additive-free $scCO_2$ step to selectively remove excess essential oil after microbial inactivation. This post-treatment effectively reduced surface residues without compromising antimicrobial efficacy, offering a viable strategy to balance safety and sensory quality (Casas et al., 2016).

Beyond food application, these additive-assisted $scCO_2$ treatments have also been successfully applied to delicate biomedical materials - including collagen scaffolds, hydrogel-based drug delivery systems, and personal protective equipment- which require low-temperature, residue-free sterilization. Such processes, achieved using food-grade additives, demonstrate the versatility of $scCO_2$ technology across multiple domains and its potential as a gentle yet powerful sterilization platform (Ruiz et al., 2025; Warambourg et al., 2023).

2.3.2. *High power ultrasounds + $scCO_2$*

The integration of $scCO_2$ with high-power ultrasound (HPU) represents a promising non-thermal approach for enhanced microbial inactivation, as demonstrated by numerous studies (Castillo-Zamudio et al., 2021; Gomez-Gomez et al., 2021, 2022; Koubaa et al., 2018; Morbiato et al., 2019). The synergistic combination of $scCO_2$ and HPU achieves substantially higher or even complete microbial inactivation, compared with either treatment alone under identical processing time. This approach underscores its potential to markedly reduce inactivation times, particularly in liquid food matrices, where it has shown superior efficacy (Paniagua-Martínez et al., 2018). The enhanced efficacy is attributed to acoustic cavitation phenomena, which increased the overall mass transfer and accelerate pH reduction owing to the greater solubilization of $scCO_2$ into the aqueous phase. Moreover, the mechanical energy generated by ultrasonic waves facilitates cell walls and membrane disruption, a mechanism that also contributes to inhibiting microbial regrowth during post-treatment storage (Ortuño et al., 2014).

Although the combination of $scCO_2$ and HPU has demonstrated significant efficacy in liquid systems, its application to solid food matrices remains limited to coriander leaves and chicken breast meat, showing a synergistic effect on vegetative cells but only modest activity against mesophilic spores (<2log inactivation on coriander leaves) (Michelino et al., 2018; Morbiato et al., 2019). This limitation primarily arise from the intrinsic constraints of HPU technology, which was originally developed for homogeneous or flowable media. To overcome this, an alternative approach has been proposed involving the immersion of solid foods in conductive liquid media (e.g., saline solutions) during treatment. This configuration enables more efficient propagation of ultrasonic waves and facilitates CO_2 penetration, thereby enhancing microbial inactivation efficiency. Preliminary studies using this strategy have yielded promising results in improving microbial safety (Castillo-Zamudio et al., 2021), yet further validation is needed to confirm its effectiveness across diverse food categories and processing conditions.

2.3.3. *Pulsed electric fields + $scCO_2$*

Pulsed electric fields (PEF) induce electroporation by applying short, high-voltage electric pulses across microbial cells, generating transient pores that temporarily disrupt the integrity of cell membranes and

increase their permeability. When used in conjunction with $scCO_2$, PEF enhances CO_2 diffusion into the intracellular space, thereby amplifying acidification and structural disruption (Pataro et al., 2014). This combined strategy is particularly effective when PEF is applied either before or concurrently with $scCO_2$, creating a transient window of vulnerability in the microbial membrane. Pataro et al. (2010) showed that *Saccharomyces cerevisiae* exposed to PEF (12 kV/cm, 20 J/mL) followed by $scCO_2$ (8 MPa, 25 °C) was fully inactivated within 30 min, whereas either treatment alone caused only negligible reduction. The authors attributed the observed synergy to PEF-induced electroporation, which facilitated CO_2 transport across the membrane, enhancing intracellular acidification and structural damage. The inactivation kinetics were modeled using the Peleg equation, demonstrating that treatment efficacy strongly depends on the electric field strength and specific energy input. Notably, variation in buffer conductivity had only a minor influence on microbial response, suggesting the robustness of the combined process across different medium compositions (Pataro et al., 2010). This approach was later extended to *Escherichia coli*, which exhibited greater log-reduction at higher electric field intensities and longer CO_2 exposure times (Pataro et al., 2014).

SEM images confirmed morphological alterations, including membrane collapse and surface wrinkling, supporting the proposed mechanism of enhanced CO_2 penetration through electroporated membranes. Spilimbergo et al. (2003) expanded this concept to spore-forming microorganisms. A PEF pretreatment (25 kV/cm, 10–20 pulses) followed by $scCO_2$ at 200 bar and 34–40 °C significantly enhanced microbial lethality. For example, *Staphylococcus aureus* and *E. coli* were completely inactivated within 10 min, whereas *Bacillus cereus* spores exhibited a 3-log reduction after 24 h at 40 °C, compared with negligible inactivation using $scCO_2$ alone. SEM observation confirmed membrane degradation in vegetative cells and surface damage in spores, supporting the mechanism whereby electroporation facilitates CO_2 penetration (Spilimbergo et al., 2003). The authors concluded that PEF pretreatment enhanced membrane susceptibility and accelerates CO_2-driven inactivation, enabling effective microbial reduction at lower temperatures and shorter processing times.

2.3.4. *High-pressure processing (HPP) + $scCO_2$*

The combination of $scCO_2$ and high-pressure processing (HPP) represents a promising strategy to overcome the inherent limitations of each technique. HPP alone typically requires pressures of 300–600 MPa (compared with 8–25 MPa for $scCO_2$ pasteurization) to achieve substantial microbial reduction. Although such high pressures make it less suitable for delicate food matrices, HPP offers the advantage of significantly shorter treatment times (<10 min compared to 5–60 min for $scCO_2$). When HPP is combined with CO_2, the inactivation threshold is significantly reduced, enabling effective microbial and enzymatic control under milder pressure conditions (Rotabakk and Rode, 2023). This synergy arises from CO_2-induced pH reduction and membrane destabilization, which sensitize microbial cells to pressure-mediated biochemical effects. These effect include the dissociation of multimeric protein complexes (e.g., ribosomes, key metabolic enzymes), inactivation of membrane-associated proteins, and induction of oxidative stress in the presence of iron, rather than mechanical collapse of cellular structures (Aganovic et al., 2021; Sehrawat et al., 2021). The presence of $scCO_2$ also enables microbial inactivation at lower HPP intensities (e.g., 150–300 MPa), potentially broadening its applicability to products in which excessive high-pressure would damage texture or appearance (Jadhav and Choudhary, 2024). The synergistic effect is most pronounced at intermediate pressures (350–400 MPa), where HPP alone might already achieve moderate log reductions, while the addition of $scCO_2$ further enhances microbial lethality and storage stability (Rotabakk and Rode, 2023). At these pressure levels, $scCO_2$ proved highly effective against *Listeria monocytogenes*, suppressing post-process regrowth for more than 45 days under refrigerated storage. This funding suggests a dual-action mechanism, involving immediate structural disruption and prolonged metabolic inhibition. Such interactions are particularly relevant for refrigerated foods, where pathogen recovery during storage represent a major safety concern (Yang et al., 2023).

Beyond microbial inactivation, the combined treatment also provides notable quality benefits, particularly for seafood, where pressure-sensitive attributes are critical. In *coho* salmon, treatment at 150 MPa in the presence of 100 % CO_2 reduced proteolytic enzyme activity (e.g., collagenase) by >90 %, while simultaneously limiting trimethylamine formation, lipid oxidation, and color degradation (Perez-Won et al., 2020). Despite the moderate pressure level, product shelf-life was extended by at least two weeks. Nevertheless, certain enzymes, such as cathepsins, retained partial post-treatment activity, indicating that not all spoilage pathways are equally suppressed. Moreover, Pérez-Won et al. (2021) observed that combining PEF, $scCO_2$, and HPP, either sequentially or simultaneously, provided additional microbial control without compromising sensory quality, particularly in fish products (Pérez-Won et al., 2021). Although the study primarily examined the PEF + $scCO_2$ combination, the inclusion of HPP demonstrated that multistep hurdle strategies can enhance efficacy, particularly when short $scCO_2$ residence times or mild pressures are applied.

Overall, the HPP + $scCO_2$ combination appears particularly suitable for delicate food matrices, such as seafood, fermented products, or beverages, where a full thermal or high-pressure load could compromise sensory integrity. However, industrial implementation remains limited, with relatively few studies addressing fermented or ready-to-eat (RTE) products under real processing conditions (Bigi et al., 2023). Process outcomes remain strongly dependent on both the food matrix and the microbial strain, and further optimization is needed to define optimal pressure, temperature, and CO_2 phase parameters across product categories.

3. Current applications

Only a limited number of industries have begun developing inactivation and sterilization processes based on $scCO_2$, with even fewer efforts specifically targeting food applications. Although $scCO_2$ pasteurization share equipment and operational similarities with well-established extraction systems, its industrial adoption for food processing remains limited. To date, most research has been conducted at the laboratory scale, focusing primarily on low-temperature pasteurization and related applications (Pravallika et al., 2023; Zambon et al., 2022a). Existing industrial applications are mainly restricted to spin-offs and start-ups targeting specific, high-value niche products.

The scalability of $scCO_2$ pasteurization appears promising, as evidenced by successful pilot trials and small-scale industrial implementation. Its effectiveness, especially for liquid food products, has been demonstrated, underscoring its potential as a non-thermal alternative to conventional pasteurization methods (Silva et al., 2020). The growing body of scientific publications and research projects further demonstrates the rising interest and confidence in $scCO_2$ technology.

Looking ahead, the technology readiness level (TRL) of $scCO_2$ pasteurization is expected to progress steadily, driven by increasing consumer and industrial demand for gentle, sustainable food preservation methods. The $scCO_2$ approach holds particular promise for high-value categories, such as juices, dairy, meat, plant-based beverages, and solid RTE foods. Such developments could ultimately facilitate broader industrial adoption across the food sector. Another promising avenue involves the use of $scCO_2$ technology to produce postbiotics with functional and bioactive properties. This is achieved by inactivating probiotic cultures while enhancing the release of soluble bioactive factors, thereby offering potential health benefits through novel mechanisms of action (Veiga et al., 2024).

4. Challenges and perspective

Supercritical carbon dioxide treatment offers significant promise as

an alternative to conventional microbial inactivation methods. Nevertheless, its widespread industrial adoption remains constrained by unresolved technical, operational, economic, and regulatory challenges.

From a technical standpoint, the effectiveness of $scCO_2$ strongly depends on the food matrix composition and on the selected process parameters, such as temperature, pressure, and holding time (Veiga et al., 2024). Historically, the limited use of systematic and statistically robust experimental designs has hindered the clear identification and quantification of these dependencies.

A major limitation in the current body of research is the lack of methodological consistency. Many studies are restricted to narrow parameter ranges, focus on a single food matrix or microbial strain, and often lack replication and standardized controls. This heterogeneity complicates cross-study comparisons and hampers the development of predictive models and scalable operational protocols.

Beyond methodological inconsistency, the absence of validation studies using pathogen cocktails representative of relevant foodborne microorganisms remains a critical barrier to industrial implementation. For comparison, the regulatory approval and commercial expansion of HPP were achieved only after extensive microbiological validation studies with *Listeria monocytogenes* strain cocktails in RTE foods (Koutsoumanis et al., 2022; Wiśniewski et al., 2023). Current $scCO_2$ research typically relies on individual laboratory strains or non-pathogenic surrogates, often tested under simplified laboratory conditions. This reflects a historical gap between engineering-oriented process development and food microbiology, as well as the limited availability of biosafety-level 2 facilities equipped for high-pressure CO_2 operations. Bridging this gap through interdisciplinary collaboration, meta-analyses, and standardized challenge-test protocols is essential to achieve the microbiological validation required for regulatory acceptance.

Most existing research remains at laboratory scale, highlighting the technological challenges of process scale-up. Validation of $scCO_2$ processes at pilot and industrial scale is crucial to demonstrate consistent microbiological safety, sensory quality, and consumer acceptance. Although $scCO_2$ extraction systems are well established industrially, adapting similar applying similar equipment for microbial inactivation introduces distinct design and operational challenges.

From an operational standpoint, integrating $scCO_2$ into food processing requires tailored approaches depending on the product type. For liquid foods such as juices, milk, and plant-based beverages, continuous or semi-continuous flow systems can be adapted from existing extraction units, ensuring homogeneous CO_2–product contact and shorter treatment durations. Conversely, solid or heterogeneous foods pose greater diffusion barriers, often requiring longer exposure times or process modifications to avoid post-process contamination. Recent works involving the treatment of pre-packaged solid foods in non-permeable packaging, have also proven effective at laboratory scale (Spilimbergo et al., 2017; Zambon et al., 2022b). However, scale-up trials revealed inefficiencies due to unfavorable CO_2-to-surface-volume ratios (Zulli et al., 2024, 2025). Additionally, long-term shelf-life validation and quality studies remain urgently needed.

Economic factors strongly influence industrial adoption of $scCO_2$. Despite the food sector's familiarity with CO_2 extraction systems, the economic feasibility of $scCO_2$ microbial inactivation across market segments remains uncertain. Comprehensive cost-benefit and profitability assessment are therefore essential. Recent case studies on fruit juice pasteurization (Gallinaro et al., 2021) demonstrated that $scCO_2$ processing can be economically sustainable over a 10-year period, with positive net present value (NPV) and internal rates of return exceeding the cost of equity, even under conservative market assumptions. Shared industrial facilities offering modular batch or systems could further reduce financial barriers and facilitate collaborative investment, process optimization, and workforce training. Continuous processing systems, characterized by higher throughput and shorter cycle times, remains particularly attractive for improving economic viability and scalability.

Synergistic combinations of $scCO_2$ with other non-thermal technologies hold great potential but remain underexplored. As noted above, well designed, standardized experimental frameworks are essential to reliably quantify and optimize these synergistic interactions. Moreover, hurdle-based combinations may help reduce the formation of sublethal injuries or viable-but-non-culturable (VBNC) cells, which otherwise compromise inactivation efficacy and complicate shelf-life predictions. Exploring sterilization-level treatments through combined hurdle technologies or co-solvents systems could significantly broaden the commercial appeal and market reach of $scCO_2$, enabling the production of shelf-stable foods with lower energy and storage costs.

Environmental sustainability is often cited as a potential advantage of $scCO_2$ technology, owing to its lower energy use and minimal waste generation. However, comprehensive life cycle assessment (LCA) studies are still required to quantitatively evaluate the environmental performance of $scCO_2$ processes relative to conventional thermal treatments.

Finally, regulatory uncertainty remains a major obstacle. In Europe, classification of $scCO_2$-treated foods as "novel foods" under Regulation (EU) 2015/2283 may triggers lengthy approval procedures, particularly when nutritional, sensory, or structural properties are significantly altered. Typically, $scCO_2$ processes cause minimal modification of these attributes compared to thermal treatments. Moreover, residual CO_2 can qualify as a technological adjuvant under European Regulation (EC) 1333/2008, potentially exempting treated products from labeling requirements. Internationally, regulatory frameworks remain fragmented, and the lack of harmonized standards could further limit global adoption. Efforts to align international regulation and promote mutual recognition would facilitate broader acceptance of this technology.

5. Conclusions

Supercritical carbon dioxide ($scCO_2$) technology is a promising non-thermal alternative to traditional preservation methods, achieving effective microbial inactivation while maintaining sensory and nutritional quality. Inactivation mechanisms include pH reduction, membrane disruption, and enzyme inhibition, which together target a broad range of microorganisms and can inactivate spores when combined with additional hurdles. However, efficacy varies by food matrix and microbial species, and the presence of sublethal injuries or VBNC cells raises concerns regarding post-treatment regrowth. Future research should prioritize microbiological validation, focusing on spore resistance, efficacy in low moisture foods, strain-specific kinetics, and VBNC cell formation and recovery. Detection and management of sublethally injured cells are essential to ensure reliable industrial outcomes. Adoption barriers, including high equipment costs, limited process standardization, and regulatory uncertainty, must be addressed thought coordinated efforts among academia, industry, and policymakers.

Progress will depend in improving cost effective, validation continuous processes, and establishing standardized operational parameters across food category. Integrating $scCO_2$ with other mild preservation technologies such as HPP, PEF, ultrasound, or natural antimicrobials offers a practical path toward sterility while preserving quality. Environmental advantages, including lower energy use and potential CO_2 reuse, further strength the appeal of this approach. Nevertheless, comprehensive life cycle assessment (LCA) studies are still lacking, and without quantitative evidence on the true environmental footprint of $scCO_2$ processing, its sustainability advantages remain largely theoretical. In summary, despite current challenges, the unique advantages of $scCO_2$ technology, supported by growing scientific evidence and industrial interest, highlight its potential as a key enable of next generation, sustainable food preservation. By addressing consumer demand for safe, minimally processed, high-quality foods, $scCO_2$ is well-positioned to drive future innovation in the food industry.

CRediT authorship contribution statement

Pietro Andrigo: Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Sara Spilimbergo:** Writing – review & editing, Supervision, Conceptualization. **Alessandro Zambon:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

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

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