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Dense Phase Carbon Dioxide: An Emerging Non Thermal Technology in Food Processing

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

DPCD is an emerging non-thermal process which evidently inactivates certain micro organisms and enzymes at lower temperatures and high pressure combination thereby maintaining fresh like characteristics as well as providing convenient shelf life, nutritional value and the product. DPCD ensures minimal nutrient loss and better preserved quality attributes as compared to any thermal treatment. This technology has been under investigation into past few decades and various studies have been carried on to demonstrate its mechanism and effect on various microorganisms such as bacteria, yeast and moulds. Many liquid foods have shown promising results. This article is a review of effect of DPCD on the quality and safety of foods with special application of juices, beverage and dairy industry.

Keywords: DPCD; non thermal treatment; shelf life; nutritional value.

1. INTRODUCTION

Non-thermal food preservation techniques have been recent trend owing to consumer demand for fresh-like food products. Thermal pasteurization is the most common method to prevent microbial spoilage of highly acidic juices. However, it can cause degradation of flavor,

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nutrient, color and texture. Dense phase CO_2 is a promising non-thermal process to preserve mainly liquid foods. CO_2 at ambient pressure can inhibit microorganisms but, when it is applied for elevated pressures, it can effectively inactivate a number of microorganisms. A technique was proposed for collecting the content of Escherichia coli by bursting cells in liquid culture with a sudden release of pressurized Ar, N_2, N_2O or CO_2, and suggested that pressurized CO_2 could be used for the inactivation of *E. coli* [1].

Several authors worked on use of DPCD for inactivation of micro-organisms, enzymes in liquid foods and its effect on physic-chemical qualities. DPCD has been shown to eliminate vegetative forms of spoilage and pathogenic bacteria, moulds, yeasts, and can inactivate some enzymes [2]. These enzymes include polyphenol oxidase, which causes browning of fruits, juices, seafood and vegetables; pectinesterase in fruit juice, which causes cloud loss; peroxidase, which causes food discolouration; and lypoxygenase, which destroys chlorophyll and contributes to the development of off-flavours in frozen vegetables. It was reported that high-pressure CO_2 had a significant effect on the inactivation of polyphenol oxidase in apple juice maintaining only 38.5% residual activity at 30 MPa and 55°C for 60 min [3].

Microbial inactivation by DPCD is dependant on different parameters like exposure time, pressure, temperature, pressure cycling, initial pH of medium, water activity, cell growth phase or age, species of microorganisms, and type of treatment system [4,5,6,7,8]. Most of DPCD inactivation studies currently available in the scientific literature have been performed on inoculated or spoiled foods [4,9]. High pressure facilitates CO_2 solubilization in water and penetration through cell walls, and increases density and therefore extraction power. Higher temperature enhances deactivation by increasing the fluidity of cell membranes, making them easier to penetrate, and increasing the diffusivity of CO_2. However, higher temperatures may reduce the ability of CO_2 to extract low-volatility materials and decrease CO_2 solubility in aqueous media. Depending on temperature and pressure, CO_2 exists on the gas, liquid or supercritical fluid state. CO_2 exists in its supercritical state above 31°C and 7.34 MPa. Below this critical temperature–pressure combination, CO_2 exists on the subcritical liquid or gaseous state depending on the specific temperature–pressure combination. Supercritical CO_2 has properties of both liquid and gaseous CO_2 with altered viscosity, diffusivity and solubility, resulting in improved dissolving power. The gas-like diffusivity allows supercritical CO_2 to quickly diffuse through complex matrices; and the liquid-like density confers high extraction power. CO_2 is having relatively low critical pressure (74 bar) and temperature (32°C), relatively non-toxic, non-flammable, available in high purity at relatively low cost, and is readily available. [10] reports that Baker's yeast, E. coli and S. aureus were completely sterilized by SC-CO_2 at 200 atm and 35°C while no sterilizing effect was detached with G- and L-CO_2. Experimental results have been also published showing DPCD effects on other products as dry cured ham [9], fresh cut pear [11], fresh cut carrot [8], spinach leaves [12], alfalfa sprouts [13].

2. INACTIVATION KINETICS

Lin et al. observe a two-stage kinetic curve. The first stage was characterized by a slow deactivation rate, and the second stage by fast linear deactivation. Two-stage kinetics were also observed by several other researchers (Fig. 1(a) and (b)). Some studies showed a fast initial rate followed by a slow deactivation stage (Fig. 1(d)). Interestingly, both kinds of kinetic curves appeared in [14] (Fig. 1(e)). One reason for variation in kinetic behavior may be the difference in the efficiency of contact between CO_2 and the microorganism. That depends on process temperature, pressure, state of CO_2, type of suspension medium etc.

3. DEACTIVATION MECHANISM

Physical cell rupture was the earliest proposed mechanism. Cells were presumed to rupture because of the explosive expansion of high-pressure CO_2 during flash depressurization. However, based on electron microscopy, [15] proposed that, the rupture of cells happens *during* pressurization when the cells are swollen by CO_2, rather than during the depressurization stage. The physiological deactivation mechanisms include several different scenarios,

Step 1: Solubilization of pressurized CO_2 in the external liquid phase.

Water in contact with pressurized CO_2 generally becomes acidic due to the formation and dissociation of H_2CO_3, which liberates H+ ions. This lowered extracellular pH may inhibit microbial growth [16,17,18]. However, a reduction in pH is not enough to account for the lethal effect of CO_2. [5,19] therefore suggested that the lowered pH contributes to an increase in cell permeability, which facilitates the penetration of CO_2 into microbial cells.

Step 2: Cell membrane modification.

Aqueous CO_2 may diffuse into the cellular membrane and may accumulate into its lipophilic (phospholipid) inner layer [20]. This accumulated amount of CO_2 in the lipid phase may then structurally and functionally disorder the cell membrane due to an order loss of the lipid chain (a process known as "anesthesia") [21], which may increase the fluidity and hence permeability of the membrane [20].

Step 3: Intracellular pH decrease.

Due to the increased membrane permeability, pressurized CO_2 may easily penetrate through the bacterial cell membrane and accumulate in the cytoplasmic interior of bacterial cells. There, the relative concentrations of both aqueous CO_2 and HCO_3^{-2} are in first instance controlled by internal pH buffering as a result of pH homeostasis in order to maintain a more or less constant cytoplasmic pHi. A more important homeostatic system is a membrane-bound H+-ATPase [18] which expels protons from the cytoplasm against the prevailing pH gradient and electrochemical gradient. If too much dissolved CO_2 enters the cytoplasm, the cells may be unable to expel all the resulting protons and pHi will start to decrease. If pHi is lowered too much, cell viability will seriously be impaired.

Step 4: Key enzyme inactivation/cellular metabolism.

Enzymes have maximal activity at the optimum pH, and their activity declines sharply on either side of the optimum. So lowering of the cytosolic pHi might cause inhibition and/or inactivation of key enzymes essential for metabolic and regulating processes [18].

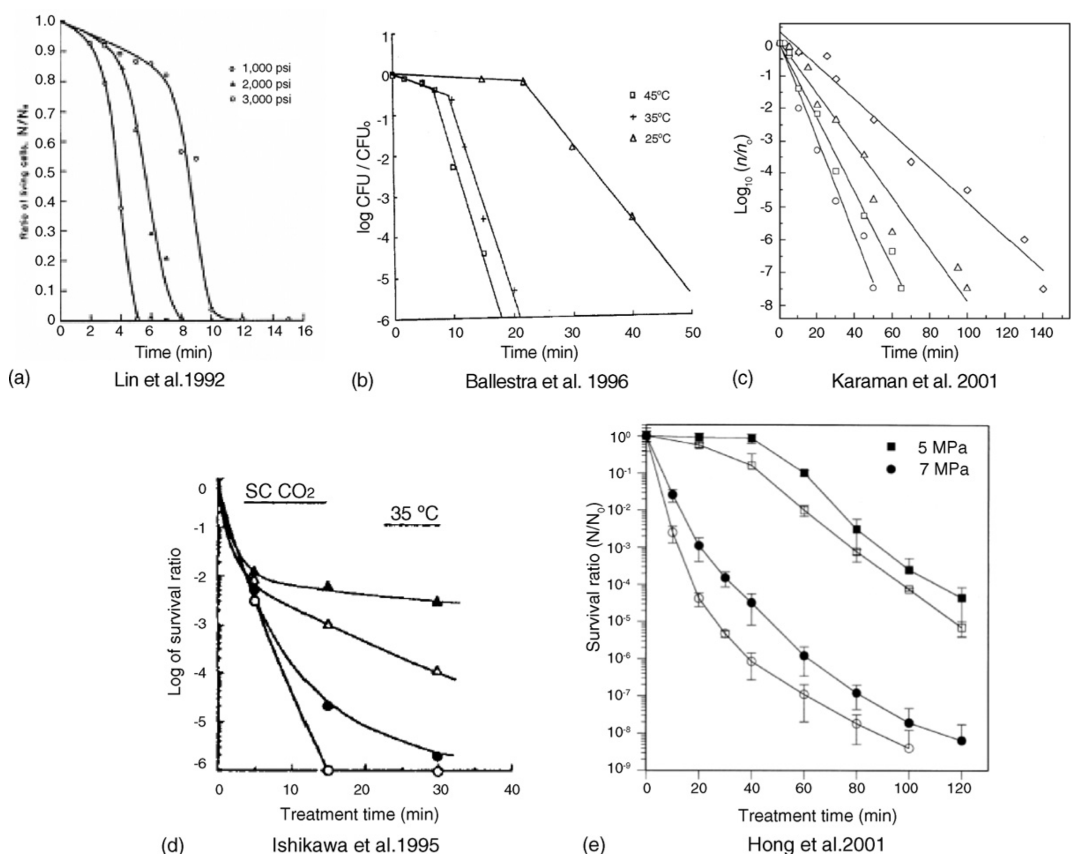

Fig. 1. Different kinds of kinetic curves reported in publications (J. Zhang et al. 2006)

Step 5: Direct (inhibitory) effect of molecular CO_2 and HCO_3^{-2} on metabolism.

The reaction rate of each enzymatic reaction is not only a function of the pH but also of the intracellular concentrations of its substrate(s), product(s), and cofactor, which are primary elements in the regulation of enzymatic activity. The concentration of HCO3 − appears to be central to the regulation of enzymatic activity (and hence cellular metabolism). Dissolved CO_2 can inhibit decarboxylation reactions.

Step 6: Disordering of the intracellular electrolyte balance.

Lethal damage to the biological system of the cells may also be produced when the applied CO_2 pressure accumulates in the cytoplasmic interior of the bacterial cells. This may convert HCO_3^{-2} to CO_3^{-2}, which could precipitate intracellular inorganic electrolytes (such as Ca^{2+}, Mg^{2+} and similar ions) from cells and cell membranes [5]. Since these inorganic electrolytes aid in maintaining the osmotic relationships between cells and their surrounding media, this could have deleterious effects on the volume of cells.

Step 7: Removal of vital constituents from cells and cell membranes.

The pressurized CO_2 first penetrates into the cells to build up the density of a critical level within the cells, after which it removes intracellular constituents (such as phospholipids and hydrophobic compounds) to disturb or alter the structure of the biomembrane and/or the balance of the biological system, thus promoting inactivation [5,22]. This removal process appeared to be stimulated by a sudden release of the applied pressure, leading to a rapid transfer of intracellular materials out of the biological system into the extracellular [5,22] also suggested that the inactivation rate could be improved by repeating the release and recharge of pressurized CO_2 in the pressure vessel during the treatment to improve transfer of intracellular materials out of the bacterial cells.

4. DPCD APPLICATIONS IN FOOD PROCESSING

This process of cold pasteurization is applied mostly to liquid foods mainly fruit juices. At high pressure, the inactivation of E. coli exposed to DPCD was dramatically increased as the temperature increased [16]. Similar results were earlier observed [7]. Higher temperatures stimulated the diffusivity of supercritical CO2 and also increased the fluidity of cell membrane to make its penetration of the cell easier [7]. DPCD increases the susceptibility of apple. It had a noticable effect on apple PME activity. Z values representing temperature increase needed for a 90% reduction of the D value and the activation energy E_a of the labile fraction of 30 MPa was found to be 22.32°C and 86.88 kJ/mol [23]. DPCD has been added to cottage cheese, ice cream, yogurt and ricotta cheese because CO2 is highly soluble in lipids and aqueous solutions. The gas decreases the growth rate of microorganisms, displaces oxygen thereby minimizing rancidity, and can be combined with barrier packaging to extend shelf life, in some cases by triple [24]. A continuous high-pressure carbon dioxide system, running at ambient conditions, was tested for its performance in reducing both natural and inoculated microbial loads. The prototype system continuously processes orange juice with carbon dioxide (CO2) at high pressures. The unit was able to cause a 5-log reduction of the natural flora in spoiled juice, and could attain a 5-log decrease in numbers of pathogenic Escherichia coli, Salmonella typhimurium, and Listeria monocytogenes. [25]. The influence of thermal and dense-phase carbon dioxide (DPCD) pasteurization on physicochemical properties and flavor compounds in melon juice was investigated. Melon juice was pasteurized using DPCD treatment and compared to a conventional high-temperature-short-time (HTST) method. The DPCD treatment was carried out using a DPCD unit (55 C, 60 min, and 35 MPa). The thermal pasteurization was performed at 90°C for 60 s with an adapted laboratory setup. The changes of pH and organic acid and sugar concentrations were not significant. There were significant differences between treatments of microbial count, vitamin C, β-carotene, and volatile compound concentrations. In general, DPCD treatment had less of an effect on the measured variables than the thermal treatment but significant enough to cause microbial inactivation and nutrient preservation [12]. Red grapefruit juice was treated with continuous dense phase carbon dioxide (DPCD) equipment to inactivate yeasts and molds and total aerobic microorganisms. A central composite design was used with pressure. (13.8, 24.1, and 34.5 MPa) and residence time (5, 7, and 9 min) as variables at constant temperature (40°C), and CO2 level

(5.7%) after experimentally measuring CO2 solubility in the juice. Five log reduction in yeasts and moulds and total elimination of aerobic microorganisms occurred at 34.5 MPa and 7 min of treatment [9]. DPCD technology was investigated for its effects on milk processing [26,27], (Tisi 2004). Depending on the purpose of the process, the effects of DPCD on milk can be desirable or undesirable. Tisi (2004) showed that DPCD-treated milk had higher lipolytic activity during storage compared with the untreated milk because of the homogenization effect of DPCD on the fat micelles. [26,27] showed that DPCD can be used for casein production, due to the pH lowering effect. The use of DPCD has advantages over a traditional process using lactic or mineral acids for casein precipitation. CO2 is removed from the system after DPCD treatment, eliminating additional treatment steps [26].

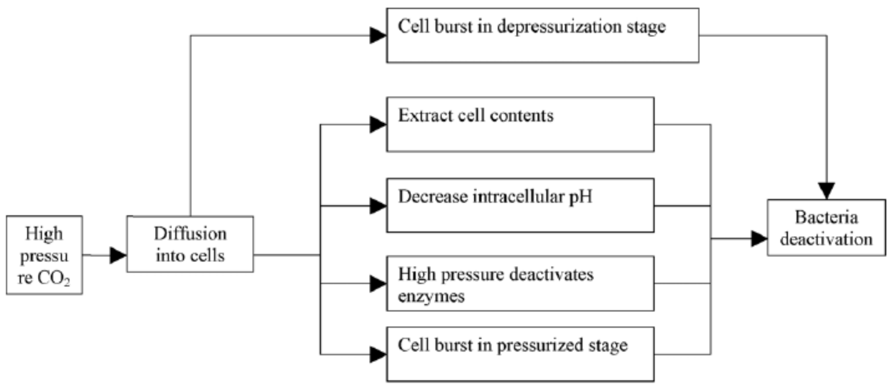

Fig. 2. Proposed deactivation mechanisms (J. Zhang et al. 2006)

Table 1. Summary of the studies on inactivation of vaious microorganisms by dense phase CO_2 (DPCD)

S. no	Solution	Microorganism	Pressure MPa	Time	Temperature (°C)	System	Log reduction	Reference
1.	Physiological saline	*Saccharomyces cerevisiae*	20	2 h	35	Batch	7.5 (C)	[28]
2.	Herbs	Total bacteria count	5.52	2 h	45	Batch	6.5 (C)	[29]
3.	Apple juice Orange juice	Total bacteria count	5.52	30 min	45	Batch	>3 (C)	
	Nutrient broth	*E. coli*			55		4 (C)	
4.	Distilled water	*Listeria monocytogenes*	6.18	2 h	35	Batch	9	[30]
5.	Egg yolk	*Salmonella thyphimirium*	2 h	2 h	8	Batch	2	[4]
	Orange juice			33 mins	35			
6.	Growth medium	*S. cerevisiae*	6.9 to 20.7	15 mins	35	Batch	7 (C)	[5,12]
7.	Growth medium	*Leuconostoc dextranicum*	4	15-20 mins	40	Batch	>8	[12]
8.	Sterile water	*S. cerevisiae*	25	< 3hrs	35	Batch	8 (C)	[31]
9.	Physiological saline	*Lactobacillus brevis*	15	30 mins	35	Batch	6 (C)	[29]
10.	Physiological saline	*S. cerevisiae*	25	30 mins	35	Micro bubble	6 (C)	[32]
11.	Sterile water	*S. cerevisiae*	15	1 hr	30	Batch	8 (C)	[26]
12.	Whole milk	Aerobic plate count	14.5	5 hrs	25	Batch	>8	[33]

5. CONCLUSION

In some years, DPCD treatment could become one of the most available emergent technologies. However, to meet this high expectation, consumers and stakeholders must be convinced about the improvements this new technology represents. This will require convincing data, and provision of clear, objective and unbiased information also including the potentially negative aspects of the technology and their limitations. HPP (pressures above 100 MPa) has been studied intensively for a number of years and continues to the present [34]. It is currently being used commercially to process higher value products that are perishable and have few processing alternatives such as guacamole and salsas. Although the FDA has not issued a final ruling, an additional benefit of using pressure to preserve foods might be to label foods as “fresh”, a term highly valued by consumers without having any adverse effects while handling and usage. Impact on green house effect can be considered to be minimal yet a study needs to be ascertained. Also, for being able to replace other preservation techniques, DPCD treatment must not only improve food quality, but also promote shelf life and (long-term) safety by inactivating spoilage and pathogenic microorganisms. Therefore, further research is essential to demonstrate and explain the effect of DPCD preservation on the shelf life and safety of food products. In addition, it is important that the effect of a DPCD treatment on the sensory and nutritional quality of both liquid and solid foods is more thoroughly investigated. Also the economics of the process must be assessed. Finally, although this review clearly shows that DPCD has great potential for improving the safety and quality of foods, some technological and regulatory hurdles (such as further optimization of the process, proper scale-up, acquisition of more sensory and shelf life data, quality certification to be obtained, etc.) still need to be overcome before the supply chain can receive these benefits.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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