Dense Phase Carbon Dioxide: A Novel Non-Thermal Technique for Inactivation of Micro-Organisms in Food

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Abstract
Dense phase CO$_2$ (DPCD) is a non-thermal technology that can inactivate certain micro-organisms and enzymes at temperatures low enough to avoid the thermal effects of traditional pasteurization. This technology has been investigated over the past 50 years, particularly in the past 2 decades, and its effects on vegetative cells and spores of various microorganisms including pathogens, spoilage bacteria, yeasts, and molds, and various enzymes of importance to foods have been demonstrated. Many liquid foods retained fresh-like sensory, nutritional, and physical properties after DPCD treatment along with some solid foods. This paper is a review of mechanisms of microbial reduction, enzyme and spore inactivation, DPCD treatment systems and examples of applications with effects on quality attributes.

Keywords: Dense phase CO$_2$; Non-thermal pasteurization; Microbial inactivation; Enzyme inactivation; Quality

Introduction

The need for a food preservation method that is safe, inexpensive, and that preserves heat-sensitive compounds resulted in the use of pressurized carbon dioxide (CO$_2$) as a food preservation method. CO$_2$ is used because of its safety, low cost, and high purity. Dense-phase carbon dioxide (DPCD) treatment has attracted great interest in the non-thermal treatment of liquid foods or liquid model solutions. DPCD has been shown to inactivate microorganisms as well as conventional heat pasteurization without the loss of nutrients or quality changes that may occur due to thermal effects. In the DPCD process, food is contacted with
pressurized sub- or supercritical CO₂ for a period in a batch, semi-batch or continuous manner. The CO₂ pressures can range from 7.0 to 40.0MPa. These levels are much lower than those of ultra-high-pressure processes. Process temperatures can range from 20 to 60°C whereas treatment times can be ranged from about 3 to 9min for continuous, or from 120 to 140min for semi-continuous or batch DPCD processes. A block diagram of batch type DPCD system is given in (Figure 1).

Inactivation of Micro-Organisms by DPCD

The mechanism of inactivation of micro-organisms by DPCD is a complex phenomenon. Several authors have given many concepts of microbial inactivation through DPCD which are summarized below [1-6].

**pH lowering effect**

It has been found that CO₂ can lower the pH of food matrix when dissolved in the aqueous part of food by forming carbonic acid, which further dissociates to give bicarbonate, carbonate and H⁺ ions lowering extracellular pH.

\[ CO_2 + H_2O ↔ H_2CO_3 \]

\[ H_2CO_3 ↔ H^+ + HCO_3^- \]

\[ HCO_3^- ↔ H^+ + CO_3^{2-} \]

A good correlation is obtained between water and liquid foods under DPCD by predicting the pH and comparing with measured pH [7]. Lowering of pH is reduced by dissolved acids and salts in DPCD. The internal pH of microbial cells is responsible for greater part of destruction of micro-organisms. With abundance in the environment, CO₂ penetrates through the cell membrane consisting of phospholipid layers and lowers internal pH by exceeding the cell’s buffering capacity. Cells are always try to maintain a pH gradient between the internal and external environment by pumping H⁺ ions out of the cell. But, the permeation of excess CO₂ into cells causes in reduction of internal pH which inactivates the micro-organisms by inhibiting essential metabolic systems including enzymes [8].

**Inhibitory effect of molecular CO₂ and bicarbonate ion**

Bacterial enzymes may be inhibited by CO₂. At low pH, protein-bound arginine may interact with CO₂ to form a bicarbonate complex, inactivating the enzyme [9,10]. Other researches show that decarboxylases are inhibited by excess CO₂ breaking the metabolic chain [11]. A complete inactivation of alkaline protease and lipase is observed at 35 °C, 15MPa using a micro-bubble system. Another proposed mechanism is precipitation of intracellular inorganic electrolytes such as Ca²⁺, Mg²⁺ and similar ions from cells and cell membranes. Since these inorganic electrolytes aid in maintaining the osmotic relationships between cells and their surrounding media, this can have deleterious effects on the volume of cells. Microbial inactivation achieved by DPCD ranges from 2 to 12 logs, using pressures below 50MPa, and temperatures between 5 °C and 60 °C, mostly in the 25 °C to 35 °C range. Treatment time is significantly different depending on the system used and can be as long as 6h with batch system and as low as 2.5min with continuous or semi-continuous system.

| Physiological saline |
|----------------------|
| Saccharomyces cerevisiae | 20 | 120 | 35 | 7.5 (C) | Kamahir et al [19] |
| Escherichia coli | 20 | 120 | 35 | 6.5 (C) |
| Staphylococcus aureus | 20 | 120 | 35 | 5 (C) |
| Aspergillus niger | 20 | 120 | 35 | 5 (C) |
| Herbs       | Total bacteria count | 120 | 45 | 5-8 (C) | Haas et al. [20] |
|------------|----------------------|-----|----|---------|-----------------|
| Apple juice| Total bacteria count | 5.52| 30 | 45      | >3 (C)          | Ballestra et al. |
| Orange juice| Total bacteria count | 5.52| 30 | 55      | 4 (C)           | Erkmen [21]      |
| Nutrient broth | *E. coli* | 6.21| 120| Room temp. | 2              | Shimoda et al. [22] |
|             | *S. aureus*     | 6.21| 120| Room temp. | 2              |                 |
|             | *Salmonella seftenberg* | 6.21| 120| Room temp. | 2              |                 |
| Distilled water | Listeria monocytogenes | 6.18| 120| 35      | 9 (C)           | Wei et al. [16] |
| Egg yolk   | *Salmonella typhimurium* | 13.7| 120| 35      | >8              | Erkmen [21]      |
| Orange juice | Total plate count (TPC) | 33   | 60  | 35      | 2               | Arreola et al. [23] |
| Growth medium | *S. cerevisiae* | 6.9 | 15  | 35      | 7 (C)           | Lin et al.       |
| Growth medium | *Leuconostoc dextranicum* | 6.9-20.7| 15-20| 35     | >8              | Lin et al.       |
| Sterile water | *S. cerevisiae* | 4   | > 180| 40      | 8 (C)           | Nakamura et al. [24] |
| Physiological saline | *S. cerevisiae* | 25  | 30  | 35      | 6 (C)           | Ballestra et al. |
| Sterile water | *E. coli*      | 5   | 20  | 35      | 6 (C)           |                 |
| MRS broth  | Lactic acid bacteria | 6.9 | 200 | 30      | 5               | Hong et al. [25] |
| TSB w/ polymers | *Bacillus cereus* | 20.5| 240 | 60      | 8 (C)           |                 |
|             | *Listeria innocua* | 20.5| 36  | 34      | 9 (C)           |                 |
|             | *S. aureus*      | 20.5| 240 | 40      | 9 (C)           |                 |
|             | *Salmonella salfood* | 20.5| 240 | 40      | 9 (C)           |                 |
|             | *Pseudomonas aeruginosa* | 20.5| 240| 40      | 8 (C)           |                 |
|             | *E. coli*       | 20.5| 30  | 34      | 8 (C)           |                 |
|             | *Legionella dunnifi* | 20.5| 36  | 34      | 8 (C)           |                 |
|             |                 | 20.5| 90  | 40      | 4 (C)           | Dillow et al.    |
| Growth medium | *Lactobacillus plantarum* | 13.8| 30  | 30      | >6 (C)          | Hong et al. [25] |
| PS with broth | *L. monocytogenes* | 6   | 75  | 35      | 6.98 (C)        | Erkmen [21]      |
| PS          | *Enterococcus faecalis* | 6.05| 18  | 35      | 8 (C)           | Erkmen [21]      |
| Fruit juice milk | *E. faecalis* | 6.05| 180-360| 45 | 5 (C)          | Erkmen [21]      |
| Pysiological saline | *Brochothrixthermosphacta* | 6.05| 100 | 35      | 5.5 (C)         | Erkmen [21]      |
| Skinned meat | *Brochothrixthermosphacta* | 6.05| 150 | 35      | 5 (C)           | Erkmen [21]      |
| MRS broth   | *L. plantarum*   | 7   | 100 | 30      | >8              | Hong et al. [25] |
| Pysiological saline | *Salmonella typhimurium* | 6   | 15  | 35      | 7 (C)           | Erkmen [21]      |
| PS with broth | *S. typhimurium* | 6   | 140 | 25      | 7 (C)           | Erkmen [21]      |
| Whole milk  | *E. coli*       | 10  | 360 | 30      | 6.42 (C)        | Erkmen [21]      |
| Skim milk   | *E. coli*       | 10  | 360 | 30      | 7.24 (C)        | Erkmen [21]      |
| Carrot juice | Aerobic plate count | 4.9 | 10  | 5       | 4               | Park et al. [12] |

### Treatment in Semi-continuous type DPCD system

| Physiological saline | Bacillus subtilis | 7.4 | 2.5 | 38 | 7 (C) | Spilimbergo et al. [26] |
|----------------------|-------------------|-----|-----|----|-------|------------------------|
|                     | Pseudomonas aeruginosa | 7.4 | 2.5 | 38 | 7 (C) |                        |

### Treatment in Continuous type DPCD system

| Sterile water | *E. coli* | 7.5 | 5.2 | 24 | 8.7 | Sins & Estigarribia |
|---------------|-----------|-----|-----|----|-----|---------------------|
| Orange juice  | *E. coli* | 15  | 4.9 | 24 | >6  |                      |
|               | *Leuconostoc mesenteroides* | 15 | <10 | 25 | >6  |                      |
A summary of inactivation rate achieved of various microorganisms, treatment conditions and type of DPCD systems used is given in Table 1. DPCD can inactivate certain enzymes at temperatures where thermal inactivation is not effective [22]. Enzyme inactivation by DPCD can be due to many causes such as pH lowering, conformational changes of the enzyme, and inhibitory effect of molecular CO₂ on enzyme activity. A study on Pectin esterase (PE) inactivation in orange juice is done in DPCD [28]. The pH of orange juice must be lowered to 2.4 for substantial PE inactivation. DPCD lowers pH only to 3.1. Therefore, only the pH lowering effect is not sufficient to explain enzyme inactivation. The results of other researchers also support this conclusion [29].

**Applications of DPCD in Food and its Effects on Quality**

Different researchers did many works regarding the applications of DPCD on physiological, biochemical and nutrional quality of foods as well as the inactivation and death kinetics of different micro-organisms.

Some of them are mentioned below:

Cloudy apple juice is treated with supercritical carbon dioxide (SC-CO₂) and the activity of polyphenol oxidase (PPO), color, and browning degree is investigated at 8, 15, 22, and 30MPa for 60min at 55 °C during storage at 4 °C for 4 weeks [30]. The PPO activity is substantially reduced with increase in pressure. The total color difference (∆E), which is significantly less than that of untreated sample, decreased by enhancing the pressure level. Cloud retention in orange juice is increased between 446% and 84.6% with increase in pressure from 30 to 100 bar (SC-CO₂) with gaseous CO₂ and liquid CO₂ in orange juice is increased between 446% and 84.6% with increase in pressure [30]. Comparison study between effect of supercritical carbon dioxide (SC-CO₂) and gaseous CO₂ (G-CO₂) and liquid CO₂ (L- CO₂) shows that endospores of Bacillus subtilis and B. stearothermophilus are resistant to SC-CO₂.

Water content has a significant effect on sterilization process. 70 ~90% water content leads to better inactivation of Baker's yeast, Escherichia coli, Staphylococcus aureus and conidia of Aspergillus niger at 200 atmospheres and 35 °C whereas water content of 2 ~10% cannot be sterilized under the same conditions. G-CO₂ and L-CO₂ has no sterilizing effect against both wet and dry baker's yeast cells, while wet E. coli cells are sterilized with G-CO₂ [24]. Treatment at 100 bar CO₂ pressure for 6h causes a decrease of 6.42 and 7.24 log cycles of E. coli in whole and skim milk [31]. Increased CO₂ concentrations and temperatures will significantly enhance the bactericidal effect, resulting in a maximum reduction of 7.31 log cfu/ml of E. coli in apple cider at 8% CO₂ and 42 °C [23]. Resistance of microorganisms to HPDCD treatment changes as a function of treatment time, leading to significant tailing in the survival curves, and is dependent on pressure and temperature.

Transmission electron microscopy shows that HPDCD treatment has a profound effect on the intracellular organization of the micro-organisms and influences the permeability of the bacterial cells by introducing pores in the cell wall [17]. Reduction in haze formation along with increase in aroma and flavor retention is found in pasteurization of beer by a continuous dense-phase CO₂ system. A maximum log reduction of 7.38 logs in yeast populations is predicted at 26.5MPa, 21 °C, 9.6% CO₂ and 4.77min residence time [32]. Similarly, a reduction of more than a 6-log cycle of yeast population is found in grape juice at the processing condition of 35 °C at a pressure of 48.3MPa with a CO₂ concentration of 170g kg⁻¹ [20]. Suspension medium like fat or oil has adverse effect on the lethality rates of bacteria in DPCD treatment system [33]. It is also showed that an increase in temperature from subcritical to supercritical of carbon dioxide leads to a significant enhancement of rapture rates of yeast cell wall under high pressures, while the functional properties of proteins and the removal of off-flavors are relatively insensitive to the variation of temperature if the process is maintained at or below 35 °C. At higher temperatures, the activities of enzymes begin to decay and are lost at about 55 °C. In comparison to conventional treatment, supercritical CO₂ has higher lethality rates for Bacillus subtilis spores at a temperature of 60 °C in 6h. Use of micro-bubble system plays a significant role for inactivation of microorganisms. Micro-bubble SC-CO₂ treatment of B. cereus, B. subtilis, B. megaterium, B. polymyxa, and B. coagulans at 40 °Cand 30MPa for 30min produces greater reduction (about 3 log cycles of reduction) than a similar treatment without a filter and with increase in temperature, the lethality rate gradually increases [34]. HPDCD treatment cannot alter the Newtonian flow behavior of the juice, for example, carrot juice, but causes a significant increase in juice viscosity (P<0.05). The browning degree (BD) and pH of HPDCD-treated carrot juice is decreased but the cloud and titrable acidity (TA) is increased significantly.
Conclusion

DPCD is an emerging non-thermal technology among all other technologies of future generations that can inactivate certain microorganisms and enzymes at temperatures low enough to avoid the thermal effects of traditional pasteurization. For that it is also called as 'Cold Pasteurization'. Retention of antioxidants, phytochemicals, organoleptic such as taste, color and appearance by DPCD system will add value to its first successful commercialization. The relatively low process temperatures, the lack of oxygen in the environment, and for some nutrients, the lower pH, protect the vitamins such as vitamin C. DPCD treatment does not only improve food quality, but also promote shelf life and (long-term) safety by inactivating spoilage and pathogenic microorganisms.

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