APPLICATION OF DENSE PHASE CARBON DIOXIDE PROCESS IN FOOD PRESERVATION

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ABSTRACT

Dense Phase Carbon-dioxide (DPCD) is a non-thermal food preservation technique for highly heat-sensitive, perishable food commodities like fruit juices, beverages, and other liquid foods. It is a continuous process, which utilizes pressurized CO_2 (pressure $1/10^{th}$ to that of the HHP) with a mild temperature of 30-50°C in food processing. At shallow temperature and pressure combination, DPCD helps to extend the shelf-life of liquid foods by destroying harmful microbes and enzymes. This helps to retain the organoleptic and quality attributes of liquid foods. DPCD is also called 'Cold pasteurization' where the CO_2 can be used either as a liquid, gaseous or supercritical fluid state. This technology can be used in combination with other non-thermal techniques like High Hydrostatic Pressure (HHP), ultrasound, irradiation and pulsed electric field (PEF). The advantages of DPCD include the shelf-life extension of food by controlling oxidation, pH, retains the food quality and the cost of CO_2 is inexpensive. The disadvantages of DPCD is a high capital investment in

commercializing the technology compared to high-volume thermal pasteurization, global warming, recycling of CO_2 during processing. The present chapter details the processing and preservation aspects of DPCD in food along with its advantages and disadvantages.

1. INTRODUCTION

Food preservation is considered as one of the most challenging and competing strategies in food processing sectors in prolonging the shelf-life of the food products and to deliver it more reliably to the consumers. Various preservation techniques like preservation by using chemicals (Class-1 preservatives: salts, sugar, oils and spices *etc*., and Class-II preservatives: benzoic acid, sorbic acid and acetic acid, *etc*.,) thermal processing (application of heat to destroy microorganisms *Ex*: pasteurization, sterilization and ultra-high temperature, *etc*.,) and non-thermal processing (High-pressure processing, pulsed electric field and ultrasound technique, *etc*.,) have been adopted to improve the quality of food products by nullifying the activity of vegetative cells of microorganisms, yeast, moulds and enzymes. Apart from all the mentioned food processing techniques, non-thermal food processing is fast emerging food processing method where the food products are subjected to a very low-temperature processing conditions to overcome the nutrient losses [40]. Dense phase carbon dioxide (DPCD) is an emerging non-thermal method of food preservation technique that utilizes high pressure or pressurized carbon dioxide (CO_2) existed in all three states, *i.e*., solid, liquid and gaseous. This method of preservation technique has been employed not only in food processing industries to minimize the nutrient deterioration in solid foods but also in pharmaceutical processing sectors [3]. DPCD can also be called ‘cold pasteurization’ which is mainly used to treat heat-sensitive food products, primarily liquid foods. The DPCD process involves subjecting the food products to a pressure range of 7.0 to 40 MPa with a process temperature of 20-60°C in a pressurized, sub or supercritical carbon dioxide in a batch, semi-batch or continuous for a specified period of time. The treatment time in DPCD generally ranges from 3-9 min. in case of constant processing and 120-140 min. in semi-continuous processing.

In case of thermal food preservation due to exposure of food products to an elevated temperature at 60 to more than 70°C leads to nutrient losses and alteration in organoleptic properties in case of both solid and liquid foods. So there is an excellent demand for preservative-free foods that retain the original freshness pave the way for the concept of non-thermal food preservation methods. In such cases, DPCD is suggested as an alternative non-

thermal cold pasteurization technique widely employed in preserving the nutritional attributes in liquid foods like juices, squashes and other liquid-based beverages that are sensitive to heat without giving up the organoleptic and nutritional properties [67]. Few pieces of research concluded the effect of DPCD against microbial activity in liquid foods. Studies conducted by few researchers confirmed the impact of DPCD in disrupting the microbial cells through decompression of carbon dioxide gas at a pressure of 500 lb.f/in^2 or about 3.45 MPa to atmospheric pressure. The process of DPCD involves subjecting the food product to a pressurized carbon dioxide that existed as supercritical or subcritical gas or sometimes liquid under pressure [28]. The effectiveness of DPCD against inactivating the enzymatic and microbial activity can be done by using pressurized carbon dioxide alone or can be combined with other gases like nitrogen *etc*., Since carbon dioxide is an inexpensive gas with a bactericidal effect can be widely accommodated in large food processing industries. There are certain advantages of DPCD over HHP as it is performed at a mild temperature condition of 30-60°C and a mild pressure of <30 MPa compared to HHP which requires a pressure range of 300-1200 MPa. The pressure needed during DPCD food processing is 1/10th of the pressure utilized in the HHP method of food processing. The other significant advantages are: it is a continuous operation; helps in the destruction of microorganisms, inactivation of enzymatic activity and nurturing the vitamin C losses are possible by reducing the pH by dissolving the CO_2. Automatic carbonation is also possible if necessary, through this method [3].

This chapter elaborates on the mechanism of enzyme and microbial inactivation by use of DPCD techniques, along with their mode of action and effects on various food products. The combination of DPCD with other non-thermal preservation techniques is also explained in further sections, elaborating on future trends exploitation of DPCD as a highly effective residue-free, non-thermal preservation technique for shelf-life extension of various food products.

2. MECHANISM OF DPCD ON FOOD AND MICROORGANISMS:

DPCD utilizes the anti-microbial characteristics of carbon dioxide at ambient or mild temperatures under pressure less than 50 MPa to extend shelf stability of sensitive foods. At such process conditions, at elevated pressure, CO_2 can exist in gaseous, liquid (< 31.1°C) or supercritical state (< 31.1°C and < 7.38 MPa), exhibiting physical properties characteristic to each form**.** At a supercritical stage, CO_2 possesses higher density, lower viscosity and very

lower surface tension, which imparts it efficient solving power and help in impregnation of complex structures. Liquid and gaseous states of CO_2 come under sub-critical condition. The density of gaseous CO_2 is directly proportional to pressure and indirectly related to temperature. However, CO_2 density remains lower in gaseous condition than in the other two states [5]. In DPCD processing, the food material is treated using super- or sub-critical CO_2 for a set of temperature, pressure and time in continuous, batch or semi-batch systems. In any DPCD system, solubilisation of CO_2 can be increased by using agitators and turbulent flow of liquid and pressurised gas. In stagnant systems, DPCD is given after the product is reached to a prescribed temperature. Whereas in continuous operation systems, the product is initially saturated with the micro bubbling of CO_2 under high pressure, after which it is passed through heater [61]. In semi-continuous processing, the fluid is made to flow continuously through pressure vessel by micro bubbling CO_2 into the product, to aid the increase in the concentration of gas pressure [66].

2.1 INACTIVATION OF VEGETATIVE MICROORGANISMS:

The combination of time, temperature, pressure and CO_2 concentration, with other factors like the purity of CO_2, pH drop *etc*., determine the microbial inactivation effect of the treatment. Previous researchers have shown a 7-log reduction in *Saccharomyces cerevisiae* at 40°C and 4 MPa pressure after three hours of exposure, while similar conditions did not affect the yeast [23]. Similarly, *Listeria monocytogenes* was reported to be completely incapacitated by CO_2 treatment at 35°C and 6.2 MPa pressure for 2 hours [73]. The bacteria are broadly categorised into gram-positive bacteria, which contains a thick peptidoglycan layer and stronger cell walls and gram-negative bacteria with complex cell wall structure and thin peptidoglycan layer. Due to the presence of peptidoglycans in the cell wall, gram-positive bacteria are comparably more resilient to mechanical stress than its counterparts. This makes gram-negative bacteria to be comparably more intolerant to DPCD treatment than gram-positive bacteria, though both types are susceptible to inactivation at high-pressure CO_2 [24].

It has been a well-known fact that CO_2 can exert inhibitory effects on bacterial growth [70]. However, the actual mechanism of action for the bactericidal effect of CO_2 has not been elaborated ever since. Several previous studies have elucidated and reviewed some major theories in this regard to explain the inhibition of microorganisms by DPCD [18,32,38,45,63]. As explained by Garcia-Gonzalez *et al*. [32], the steps involved in

bacterial inactivation mechanism by DPCD are given below. These steps may occur either in singular or consecutively, but are simultaneous and interrelated in occurrences.

- Dissolution of DPCD in the liquid matrix of food components
- Alteration of cell membranes due to pressure
- pH reducing activity
- Effect of molecular CO_2 and bicarbonate ions indirect inhibition of metabolism
- Breakage and mechanical damage of cell wall
- Imbalance in electrolyte balance of viable cells
- Enzyme denaturation
- Damage to cellular organelles

The cell membrane of microorganisms majorly contains phospholipids, which is more likely to be affected by CO_2 due to its lipophilic properties [39]. Due to this, the cell membrane permeability is elevated, which lowers the extracellular pH. This causes the pressurized CO_2 gas to enter into the cell and accumulates in the cell interior [36]. This accumulation can structurally and functionally disrupt the properly affected cell membrane permeability and increases its fluidity. Simultaneously, due to the chemical interactions inside the cell by both CO_2 and HCO_3^- ions causes the Lowering of pH, as the pH adjusting mechanisms of cells may fall short due to excess CO_2 concentration. The lowering of intercellular pH results in loss of cell viability, by causing protein and enzyme denaturation and retarding the metabolic processes of cells [38,47,64]. Studies on the flow cytometric analysis revealed that post-DPCD treatment, the alteration of efflux pump occurs due to enzymatic functions and pH changes, leading to microbial inactivation [42].

During DPCD, the CO_2 initially penetrates cells and accumulates due to its high solubility and low density. After building up to a critical level, it reacts with phospholipids and other hydrophobic compounds, thus creating disturbances in the balance of the cell biological system. The sudden depressurization leads to rapid transfer of cell constituents into the extracellular environment, leading to stimulation of cell inactivation. Also, CO_2 at elevated pressure can modify the cell membrane by enlarging the periplasmic spaces causing leakage of cell constituents [37,64]. One more optional mechanism for microbial inactivation by DPCD is a mechanical distraction of cells caused by elevated pressure [37,73]. Studies have demonstrated that during DPCD, few cells have completely disrupted while some cells

shown wrinkles and holes alone on the surface [23]. Some researches show that cell inactivation due to DPCD is irrespective of cell rupture [17], as some cells may be completely inactivated but may remain intact or slightly deformed [37].

Spores have been a potential food safety threat in foods, even after most of the preservation treatments. Inactivation mechanism of DPCD in the case of spores has been unknown for long. However, researchers stated that DPCD, coupled with heat treatment, was effective in inactivating the spores in foods. Previous studies on Bacillus subtilis spores, when subjected to CO_2 at 5 MPa and 80°C has shown inactivation of spores effectively. It was suggested that in the first step, the pressurised CO_2 with assistance from heat activation, would penetrate in to the cells. The second step involves inactivation of previously penetrated cells, as heat activation tends to make the cells more susceptible to CO_2-assisted inactivation [6]. Another alternative theory given by other studies states that DPCD results in the germination of bacterial spores at relatively low temperature due to elevated pressure. The germination of spores could be resulting in increased sensitivity of bacteria, leading to inactivation [29]. When compared to high hydrostatic pressure processing and heat treatment, DPCD has a higher killing effect at a comparatively lower temperature, owing to its raised pressure [72].

2.2 INACTIVATION OF ENZYMES

DPCD treatment has also been reported to be effective in enzyme inactivation, especially in fruits and vegetables where enzymes are the major reasons for quality deterioration. The key cause for enzyme inactivation is the pH lowering effect of extracellular solutions as described above. Nevertheless, the rate of enzymatic degradation does not entirely depend on pH, but also is affected by the intracellular concentration of cofactors and substrates and products that act as key elements for controlling the enzymatic activity [41]. The lowering of pH also disturbs the ionic equilibrium and volume of the cells [30]. The drop on pH causes the conversion of HCO_3^- to CO_3^{2-} ions, which can form complexes by binding to inorganic electrolytes like calcium and magnesium. These complexes can alter several regulatory activities of the cells and impair the protein function [36]. Previous studies have demonstrated that CO_2 at high pressure can cause spatial and conformational alterations in secondary and tertiary protein structures [60]. It has been demonstrated that DPCD has caused decomposition of α-helix structures, whereas changes in secondary structures only caused a reduction in activity [15,68]. The spatial changes in

protein due to DPCD can be either reversible or irreversible is reliant on the pressure applied. At pressure lower than 310 MPa, the change is reversible upon depressurisation, whereas below that the alterations are permanent and cannot be reversed [15,35]. CO_2 in the super-critical state has been reported to alter isoelectric profile and structure of polyphenol oxidase protein, but no changes were reported at atmospheric pressure [15].

3. DENSE PHASE CARBON DIOXIDE (DPCD) IN FOOD PROCESSING AND ITS APPLICATIONS

The usage of DPCD was first patented by Swift and Co., in 1969 in food product pasteurization using CO_2 at super atmospheric pressures. In 1980, other researchers affirmed the bacteriostatic and inhibitory effect of CO_2 on growth and microbial multiplication in food products. From the studies, it was proven that *Pseudomonas* was found to be very sensitive to CO_2 gases compared to other microorganisms like *Lactobacillus* and *Clostridium*. Other researchers conducted the sterilizing effect of CO_2 on *Escherichia coli* (both wet and dry), *Staphylococcus aureus* and conidia of *Aspergillus niger* with the help of supercritical fluid extraction equipment. Application of DPCD in the preservation of liquid food products was well renowned, but still some research works have to be done in the preservation of solid foods. The difficulties in preserving the solid food product using DPCD is the slow diffusion of CO_2 in the bulk of solid foods leading to cellular damage with undesirable surface texture which causes severe quality-related issues, limited CO_2 solubility due to decreased levels of free water on the surface of the food in connection to bacterial and enzymatic inactivation. The brief explanations on solid food preservation using DPCD are mentioned in the upcoming sections of the chapter.

3.1 APPLICATION OF DPCD IN THE PRESERVATION OF LIQUID FOODS

From the nineteenth century, it was well known that CO_2 gas is more likely to be dissolved in liquids rather than in solids. The saturated solution formed after dissolving CO_2 is the analytical composition that can be expressed as an amount of pronounced solute in a solvent. As we know that CO_2 is widely used in food processing industries due to the following benefits like nontoxic, non-combustible, less expensive and it is considered as ‘Generally regarded as safe’ (GRAS) by USFDA. Dense phase CO_2 in its collective form acts as an anti-microbial with enzyme inactivation properties at elevated pressures. The major attributes that should be taken into consideration in liquid foods are physical (colour,

viscosity and brix, *etc*.,) and chemical properties (pH, water activity, volatile components). Liquid foods are highly heat-sensitive, which losses its organoleptic properties when exposed to elevated temperatures. A great number of studies reported the use of DPCD applied on fruit juices and other non-fruit beverages. The compilation of different fruit beverages treated with CO_2 in response to different treatment conditions, initial microbial population, target organisms, and enzymes inactivation, is given in Table 1.

The foremost utilization of DPCD in the processing of liquid foods is microbial inactivation and apoptosis. Other reasons like enzyme inactivation, improvement of physical quality of foods and other chemical attributes. Park *et al*. [54] studied that treatment of DPCD on carrot juice resulted in a 60% cloud loss. Conflicting results were stated by Arreola *et al*. [1], Balaban *et al*. [4] and Yagiz *et al*.[75]. It was reported that the effect of DPCD had retained cloud stability in orange juices after refrigerated storage. Arreola *et al*. [1] observed that pectinesterase enzyme was restored during storage and concluded that the stability of the cloud is not due to the enzyme activity. Gui *et al*. [33] established that there was a significant drop in browning of cloudy apple juice when treated trials were stored at 4°C as compared to untreated samples. The reason for this can be the inactivation of polyphenol oxidase enzyme due to the treatment. Park *et al*. [54] evaluated the shelf life of carrot juice treated with DPCD treatment in combination with high hydrostatic pressure. After a shelf life of 4 weeks at 4°C refrigerated storage, treated samples showen no viable aerobic bacterial count, while control sample shown 8.4 log cfu/ml. Kincal *et al*. [43] studied that DPCD treatment at different pressures inactivated *S. typhimurium* effectively showing no viable count till 14 days of storage, without heat pasteurization methods. Del Pozo-Insfran *et al*. [57] studied comparable microbial viability amongst heat-treated and DPCD treated samples of muscadine grape juices at refrigerated storage for five weeks. It was reported that yeast count in DPCD samples consistently increased with storage time, whereas heat pasteurized samples had no change in the count of total aerobic microbes.

Previous studies have also shown that DPCD treated samples did not significantly differ from non-treated samples. Several researchers evaluated the loss of sensory and nutritional properties to assess consumer perspectives using difference and ranking tests. Balaban *et al*. [4], Lecky and Balaban [44], Arreola *et al*. [1], Damar and Balaban [18] expressed that DPCD treated samples shown no significant variance in flavour, aroma and overall consumer acceptability in comparison with fresh controls. In a few studies, DPCD

treated samples were rated higher than heat pasteurised samples [18,57]. Arreola *et al*. [1] have reported that ascorbic acid retention was most elevated in DPCD treated orange samples. At the same time, Del-Pozo Insfran *et al*. [57] evaluated that DPCD muscadine grape juice shown no substantial changes in total anthocyanins, total phenolics and antioxidant capacity when compared to 16%, 26% and 10% decrease in heat-treated samples respectively. It was also stated that anthocyanin content and antioxidant capacity of DPCD samples were better than heat-treated samples even after ten weeks of storage at 4°C. Chen *et al*. [14] evaluated that DPCD treated melon juice has retained a better amount of volatile compounds, carotenoids and ascorbic acid, whereas heat-treated samples shown significant losses and produced an unacceptable cooked off-flavour.

TABLE 1. MICROBIAL INACTIVATION IN DIFFERENT BEVERAGES THROUGH DPCD.

Sl. No	Fruit Juice	Microorganisms/ enzymes Responsible for spoilage	Quality Parameters before processing	DPCD Processing pressure	Processing temperature and time	Output after DPCD processing	Shelf-life	References
1.	Valencia orange Juice (VOJ)	Pectin methylesterase enzyme (PME) • Causes loss of cloud	Colour, pH, Brix cloud stability, colour, ascorbic acid content, and sensory properties	7-34 MPa	35-40°C for 15-18 min.	• No significant difference in pH, Brix • Better retention of Vitamin C compared to control samples.	66 days under refrigeration condition	[1]
	Orange juice (OJ) (*citrus sinensis*)	Pectin methylesterase enzyme	Particle size, consistency coefficient and *a** values	40 MPa	55°C for 10-60 min.	Reduction in particle size increased cloud stability due to homogenization during DPCD treatment	-	[50]
			Volatile components (ethyl butyrate, trans-hexanol, ∝-pinene, phellandrene, limonene,	-	10-60 min. of DPCD treatment	• Linear decrease on ethyl butyrate and trans-2-hexanol • Increase in nonanal and citronellol	-	

			linalool, nonanal and citronellol			compared to untreated OJ		
2	Apple Juice	Enzymatic browning reaction	Browning index	30 MPa	55°C for 60 min.	Degree of browning rection was significantly reduced	-	[51]
	Apple slice	Enzymatic browning Polyphenol oxidase (PPO) and pectin methylesterase (PME)	Colour and textural changes	20 MPa with mild heat	25-65°C for 20 min.	Complete inactivation of PPO and PME.	-	[25]
	Apple puree	Microbial spores	pH, °Brix, colour, microbial load	16.0 MPa for 40-60 min.	Thermal process (35, 50, 65 and 85°C) for 10-140 min.	• 5-log reduction destruction in microbial spores • No significant difference in °Brix • Significant changes in pH, colour	-	[33]
	Apple juice	Alicyclobacillus acidoterrestris spores	Physicoche mical properties like colour, sensory, pH and °Brix	80, 100 and 120 bar for 10-40 min.	65-70°C	Surface and internal morphological changes • Complete reduction of *A.acidoterres*	-	[2]

						tris spores after DPCD		
3.	Mandarin juice (*Citrus reticulate Blanco*) (Murcott cultivar)	Total aerobic count, Pectinesterase activity, cloud, °Brix, pH and titratable acidity	Temperature, pressure and residence time, pH, °Brix, colour change, titratable acidity (TA)	41.1 MPa, 9 min. and 7% CO_2	35°C	• Retention of cloud • Increase in yellowness and lightness • Reduction in redness • Increase in cloud • Brix, pH and TA were the same before and after DPCD processing	-	[46]
4.	Grapefruit juice (V. Red blush) (*Citrus paradisi*)	Yeast and mould	pH, °Brix, TA, Phenolic content	13.8, 24.1 and 34.5 MPa, 5.7% CO_2	40°C for 5, 7 and 9 min.	• 5-log reduction in total aerobic MO's including yeast and mould at 34.5 MPa for 7 min. • Increased cloudiness (91%) • Inactivation	6 weeks at the refrigerated condition	[26]

						of pME (69.17%) • No noteworthy difference in Brix, pH and TA • Phenolic content: unaffected • Slight difference in Ascorbic acid		
5.	Water melon juice (*Citrullus lanatus*)	Aerobic Microorganisms	pH, °Brix, TA, lycopene and colour	10.3, 20.6 and 34.4 MPa, CO_2: 5,10 and 15%	30-40°C for 6 to 46 min	• 6-log reduction in native MO's at 34.4 MPa, 40°C, 10% CO_2 level for 5 minutes • 4.5 log reduction of MO's in acidified, sweetened and carbonated products	Up to 8 weeks under refrigerated conditions	[44]
6.	Coconut water (*Cocus*	Decomposition of aroma components	Likeability and flavour	34.5 MPa, 13% CO_2	25°C for 6 min.	Better retention of aroma, taste and overall flavour	-	[19]

	nucifera)					compared to heat treatment		
7.	Guava puree (*Psidium guajava L.*)	Phytochemicals, Ascorbic acid, pH, °Brix, % TA, sensory and nutritional attributes	Undesirable changes in phytochemical composition, measurement and comparison of chemical compounds, microbial reduction	34.5 MPa, 8% CO_2	35°C for 6.9 min.	• No changes in pH, °Brix • Increase in TA and viscosity • Partial inactivation of PE • Protects polyphenolic and antioxidant levels • Delays vitamin C degradation which is highly heat sensitive	14 weeks at 4°C	[60]

3.2 OTHER DPCD PROCESSED BEVERAGES

Other than fruit-based beverages, liquid foods like beer, milk and milk products have also been a matter of interest for several researchers in recent times. Folkes [27] have reported that DPCD aided pasteurization of beer samples have shown a 5-log or more reduction in yeast population. Though there was some stripping of flavour and aroma, the sensory panellists could not detect excessive changes in freshly treated and 30-days stored samples, and the aroma changes were not so profound to be unacceptable by the consumers. Similarly, Ceni *et al*. [12] studied the consequences of CO_2 on an endogenous milk enzyme alkaline phosphatase, a pointer of the effectiveness of the pasteurization process of milk. The research reported that about 98.2% of alkaline phosphatase inactivation rate was observed for 0.45% of CO_2 to milk ratio, continuously treated at 8 MPa pressure and 70°C for 30 min. CO_2 treatment can also be used for the extraction of specific compounds from food as it can act as a solvent in a supercritical state. This principle was used in the extraction of cholesterol from whole milk powder by treating at 20.7 MPa and 68°C for 40 min. with CO_2 flow of 6 L/min, resulting in the removal of 55.8% cholesterol with unaltered fatty acid profile, lightness and solubility index of the sample [16]. Similarly, several other non-fruit beverages like fermented drinks and dairy products have been reported to have positively benefitted by DPCD treatment and are listed in Table 2 precisely.

TABLE 2: EFFECT OF DPCD ON NON-FRUIT BEVERAGES

Sl.No.	Beverage	Causes of spoilage	Quality Parameters before processing	DPCD pressure	Exposure time and temperature	Output after DPCD processing	
1.	Beer	Microorganisms, yeast and moulds which causes off flavour	Physical and sensory attributes	27.6 MPa	21°C, 5% CO_2	• Better retention of aroma and flavour • Retention of freshness after storage period of 1 month at 1.7°C	[17]
2.	Kava Kava (*Piper methysticum*) Traditional Polynesian beverage	Bacteria (causes alteration the organoleptic properties)	Active compounds kavalactones, δ −lactones and 5, 6 dihydro δ −lactones	34.5 MPa	13% CO_2	• Change in initial pH from 6.3 to 5.5 • Change in mouthfeel • log microbial reduction from 4.0×10^5 to 1.0×10^2	[60]
3.	Jamaica beverage (*Hibiscus*	Yeasts and moulds which alters the overall	Anthocyanins and phenolic compounds	30.8 to 31.0 MPa	6.8 min.	• retention of overall quality of	[58]

	sabdariffa)	acceptability of the product during storage				the product • lesser loss of anthocyanin pigment • no significant changes in phenolics and antioxidants during storage interval	
4.	Raw skimmed milk	*Pseudomonas flourescens* that affects the storage stability and causes spoilage	Milk fat content and freshness	20.7 MPa	35°C for 10 min.	• 5.02 log reduction and bacterial inactivation	[74]
5.	Raw milk	Alkaline phosphatase enzyme activity, causes putridity and spoilage	Inactivation of alkaline phosphatase enzyme	8 MPa, 0.45% (w/w) concentration of CO_2	70°C for 30 min.	• 98.2% inactivation rate of alkaline phosphatase	[12]

4. EMERGING TRENDS IN APPLICATION OF DPCD IN THE PRESERVATION OF SOLID FOODS

In liquid foods, the effectiveness of DPCD treatment greatly depends on the mode of operation, as pressure and mode of CO_2 supply decide the level of saturation and microbial inactivation directly. In liquids, the saturation can be controlled by using atomizers or micro-bubbling systems to disperse the CO_2. This can reduce the desired exposure time significantly, thus accelerating the inactivation kinetics [71]. But in solid foods, the CO_2 is in direct contact with the substrate. This eliminates the need for elevating the pressure or saturating the medium. The required exposure time will entirely be depending on the resistance offered by the microbial strains against inactivation. This brings the temperature to be the critical process parameter and inactivation of microorganism or enzyme depends on the optimum time-temperature combination, rather than depending on all process parameters [61]. Compared to liquid foods, usage of DPCD in treating solid food materials is difficult. The complex structural arrangement of solid components and lesser moisture content in solid foods limits the solubilisation capacity of CO_2, thus affecting its bacterial and enzymatic inactivation. Also, the complex matrix in solids slows down the diffusion of CO_2 into the bulk and may lead to quality deterioration in textural and sensorial properties of foods. However, the recent trend towards non-thermal food processing strategies has increased the importance to DPCD techniques, and several studies are being done in solid food products particularly to enhance the shelf-stability without negatively impacting on the physio-chemical, nutritional and sensory properties. In most of the previous studies, solid foods were treated using DPCD by the semi-continuous or batch type system, since maintenance of high pressure in a continuous system for solid foods was not feasible. However, due to excess pressure application during DPCD treatment, there is a high probability of changes in textural and sensory properties of solid foods. Hence, it is suggested to treat the food products using DPCD at lower pressures to preserve the organoleptic properties. The following sections will detail briefly on the recent trends and application of DPCD on solid foods.

4.1 FRUITS AND VEGETABLES

One of the most critical applications of the use of DPCD in solid foods is to use in the inhibition of moulds on whole or minimally processed fruits or vegetables. Previous studies obtained a 3.5 and 1.5 log reduction for *E.coli* and *S.cervisiae* using 5 MPa pressure respectively at room temperature for 60 min. [20]. Valverde *et al*. (2010) reported a log reduction of 0.5 scales in inactivation of *S.cerevisiae* in minimally processed pears by using 6

MPa pressure and 25°C temperature for 10 min. and also observed that yeast survival rates were inversely proportional to the temperature rise [71]. The effect of DPCD on yeasts and moulds on sliced carrots were reported to be sufficient inactivation at a rate of 1.25 log cycles at 5 MPa and 20°C for 20 min. [7]. Studies by Garcia-Gonzalez *et al*. [31] informed the yeasts have lesser sensitivity towards high-pressure CO_2 than bacterial strains in foods. They also reported that the pH of foods could directly impact on the resistance of microbial strains against DPCD treatments. DPCD treatment with elevated temperature facilitates an increase in diffusivity of CO_2 and fluidity of cell membranes, thus causing metabolic reactions that lead to cell apoptosis [32]. DPCD treatment on fresh spinach leaves shown a considerable decrease in the microbial count, but the treatment adversely affected the texture and colour of the leaves became undesirable [76]. Several other authors mentioned positive results for foods like strawberries and cucumbers, but also severe tissue destruction leading to colour loss, sogginess that are critically rejected by consumers.

4.2 MEAT AND MARINE PRODUCTS

The usage of DPCD technique on meat and marine foods has recently been the area of interest for several researchers, mostly due to its ability to extract the fats at lower temperatures and to remove cholesterol [13]. It has also been used to find the effectiveness of microbial inactivation to persuade pasteurisation. Previous studies shown that in chicken strips inoculated with microorganisms, 2 log reduction was obtained after 2 hours of treatment at 14 MPa pressure and 35°C temperature [73]. Effect of DPCD on raw pork meat treated for 60min at 25°C and 6 MPa pressure achieved about 2 log reduction of spoilage organisms. However, it was observed that the treatment increased lightness in the colour of meat, giving a cooked appearance, which was unacceptable by the consumers [10]. Other studies on surimi shrimp gel treated with DPCD at elevated temperature and pressure resulted in a reduction in α-helix of myosin of protein and increase in β-sheet which in turn raises the gel strength of the material [34]. Similar results were reported by Rao *et al*., [59] in meat sausages treated with DPCD at mild temperatures of 50 to 60°C. A most recent study on oysters treated with DPCD evaluated the inhibition effects of supercritical CO_2 on the bacterial pathogens trapped in the its digestive system successfully [48]. The impact of DPCD in chemical residue-free and efficient preservation technique for improving shelf stability of meat and meat products is an innovative and beneficial technique. However, it can cause lightening of meat surface or give a cooked appearance to meat. It can also cause

oozing out of fats from meat cuts which can result in loss of flavour and texture, thus decreasing the consumer acceptability [62].

4.3 SPICES AND OTHER FOODS

The effect of DPCD as a cold or warm pasteurization method for several low-moisture foods has been successfully proven in many studies. A study shown that use of DPCD on paprika powder for 45 min. of exposure time has resulted in 2 log reduction at 80°C and up to 5-log reduction at 95°C, showing effective bactericidal effect [9]. As an extension of this study, a study on combined effects on DPCD with oregano essential oil on paprika powder was taken [11]. The results shown the dependency of microbial inactivation due to the combined treatment, on the moisture content of the sample. In the treatment of DPCD with 2.58% Oregano essential oil, at 15% moisture content 2 log reductions was obtained in total aerobic count whereas, at 35% moisture, the log reduction was found to be 2.5 cfu/ml. Studies on the efficacy of DPCD on *Aspergillus niger* and spores of *Aspergillus ochraceus* in a non-fermented high polyphenol cocoa powder, proved that at 65°C, treatment with increased pressure from 13 to 30 MPa for 40 min. resulted in the same level of inactivation of the target organisms [8]. The study also stated that use of DPCD has shown an increase the efficiency in the extraction of butter from cocoa derivatives, with an increase in pressure applied. Another study on heavily contaminated ginseng (10^{-7} cfu/g), reported that at treatment of 10 MPa pressure and 60°C for 15 h, less than 3 log reduction was obtained which was way too less than the prescribed regulations. However, addition of ethanol/water/hydrogen peroxide has resulted in complete inactivation in only 6 h of exposure time [21]. Few studies have also explored use of DPCD in the extraction of essential oils and other extracts from food components, which are of greater economic value. Patil *et al.*, [56] reported extraction of oil from bio-algae has increases 31.37% (db) at 240 bar pressure for 60 min. under controlled flow of CO_2 when a mixture of 1:1 hexane and ethanol cosolvent is used at 12:1 ratio with algal solid ratio. It was also reported that this technique offered increase in total lipid yield and improved lipid purity along with reduction in extraction time than in conventional methods of extraction.

5. EFFECT OF DPCD IN COMBINATION WITH OTHER NON-THERMAL TECHNIQUES IN FOOD PRESERVATION

5.1 DPCD AND ULTRASOUND:

The use of DPCD in fusion with ultrasound is comparatively a relatively novel concept. Ultrasound is a highly effective technique in mixing and because of which there is a

reduction in time needed for CO_2 to reach saturation, especially during batch systems. And also, there is a possible advantage that cavitation helps in the saturation of CO_2 in the liquid form. The high-pressure region of ultrasound increases the solubility of CO_2 in the liquid by acting as the driving force for saturation. During the low-pressure cycle, the decrease in pressure makes the dissolved CO_2 to supersaturate.

Morbiato *et al*.[49] combined supercritical carbon dioxide with elevated power ultrasound for drying of raw chicken meat to increase the shelf life. The experiments were carried out at 40°C, 100 bars with pressurization and depressurization at a rate of 4 and 10 bar/min respectively and it was compared with supercritical CO_2 assisted drying and hot air oven drying technique. Among which they found that combination technique (supercritical CO_2 + ultrasound (10 W)) increased the drying kinetics and reduction in weight of 75.1% after 300 min. While in hot air oven there was a reduction in weight of 74.4% at 75°C after 420 min.

Paniagua-Martinez *et al*. [53] studied the effect of supercritical CO_2 and high-power ultrasound on inactivating the microbiota in pineapple juice in a continuous flow system. The experiment was carried out at 31.5°C and 100 bars for two different residence times 3.06 and 4.6 min. It was found that microbiota was inactivated completely with fewer quality characteristics changes. They concluded that supercritical CO_2 with high power ultrasound could be an excellent substitute for cold pasteurization of pineapple juice. Timko *et al*. [69] studied the ultrasound development of emulsions that are free from surfactants consisting of dense carbon dioxide and water. They found that emulsions with limited kinetic stability were formed at 20 kHz of pulsed ultrasound with a critical power density of above 0.05 W cm^{-3} at approximately after 2 min. In this study, they found that ultrasound can be used as surfactant-free technology for producing emulsions consists of dense phase CO_2 and water, which can be further utilized in various fields like chemicals and materials application.

5.2 DPCD AND HIGH HYDROSTATIC PRESSURE:

High hydrostatic pressure (HHP) is one of the non-thermal preservation techniques in which the food products are packed before processing and thus, eliminates the post-processing contamination. Though HHP is an effective tool for removing microorganisms in liquid foods, it does not inactivate the enzymes present in food; it increases the enzyme activity in the food. *Ex*., Polyphenol oxidase. On the other hand, DPCD is the constructive technique which inactivates the enzyme. Therefore, it is sound reasoning to combine DPCD and HHP to benefit from their key advantages. Ortuño *et al*. [52] studied the effect of

combined technology of HHP and DPCD to disable polyphenol oxidase (PPO), peroxidase (POD) and pectin methylesterase (PME) in feijoa (*Acca sellowiana*) puree. The treatments given to the puree are HHP; carbonation and HHP (HHP carb); carbonation and addition of 8.5 mL CO_2 /g puree into the headspace of the package and HHP (HHPcarb+CO_2). The samples were treated at 300, 450 and 600 MPa for 5 min. They found that the addition of CO_2 into the headspace of the packaged food which is treated with HHP boosted the inactivation of POD, PPO and PME and concluded that the simultaneous application of HHP and DPCD could improve the inactivation mechanism of enzymes with carbon dioxide concentration being a key process factor. Duong *et al*. [22] studied microbial and sensory effects of combined HHP and DPCD techniques and they found that HHP carb + CO_2 has better microbial inactivation. The sensory properties of the HHP carb +CO_2 treated sample did not alter appearance and colour while it affected the texture and unsweetened feijoa samples and also found that there was no significant difference in sweetened feijoa samples.

5.3 DPCD AND PULSED ELECTRIC FIELD:

The Pulsed Electric Field (PEF) is a processing technology which uses short electric pulses to achieve microbial inactivation of food products. These short electric pulses introduce a latent variance between the inside and outside of the membrane of microorganisms, which results in permeation of the cell membranes. PEF assist the diffusion of carbon dioxide into the microbial cell by electroporation. The protein and phospholipid bilayers of the cell membrane can be weakened due to the high voltage electric field. This makes the plasma membrane to become penetrable to small particles leading to swelling and rupturing of the cell membrane. Spilimbergo *et al*. [65] investigated the efficiency of the collective usage of PEF and High-Pressure Carbon dioxide (DPCD) on killing the gram-negative bacteria *S.typhimurium*. The PEF was applied at two different conditions (i) 1 single pulse of 1 ms length at 30 kV/cm (ii) 12 pulses of 4 ms length at 30 kV/cm. For both treatments, they have kept the samples at 12 MPa, two different temperatures (22°C and 35°C) for different treating times (0-45 min.). They have compared the inactivation kinetics of *S. typhimurium* by DPCD + PEF and only DPCD. They have found out that PEF as an assisted technique to DPCD has enhanced the inactivation kinetics and has reduced the treatment time compared to DPCD alone. This combining effect is due to the electroporation, thus causing thinning of the cell wall so that intercellular components gets dispersed. Pataro *et al*. [55] studied the effect of PEF as a pre-treatment aiding for the inactivation of *S.cerevisiae* by High-Pressure Carbon Dioxide (DPCD). Different electric field strengths (6,

9 and 12 kV/cm) and different energy inputs (10, 20, and 40 J/ml) at the pressure of 8, 11, and 14 MPa for the different treatment times ranging from 3 to 30 min. They found out that the samples treated with only PEF has shown the maximum inactivation level at 12 kV/cm and 20 J/ml with the log cycle of 0.35. While the combined process shown the inactivation level equal to 3.13 log-cycles 6 kV/cm.

6. POTENTIAL FUTURE TRENDS

Though the DPCD technique is highly efficient and several researchers have proved its application in various processes, there are three major concerns in using this technology at large scale industrial level of processing. The first and most important concern is the optimization of the use of energy dynamics and economics of the process. Since there is no current use of DPCD in mass production, the cost of equipment and operation is not optimized till date, which raises a concern to industrial applications. Also, purifying and converting atmospheric CO_2 in gaseous phase into pure, dense phase supercritical fluid is an expensive and tiring operation with a high level of difficulty. Owing to the advantages of DPCD, optimization of process parameters and energy dynamics can improve its efficiency and applicability on a larger scale with minimal losses. Also, there is a need for extensive research to study the comparison of DPCD with other non-thermal techniques like pulsed light, high-pressure processing and cold plasma in different food components to evaluate the suitability of DPCD for each product in terms of quality, safety and nutritional losses if any, sensory deterioration.

The second issue is that lack of formulation of standard regulations for treating food products. The research studies so far have evaluated and elaborated on safety regulations and process parameters in a product or process-specific manner. However, if the product changes, the regulation and process parameters need to be framed again which is a problem in large establishments. This concern can be handled by predicting and developing models for DPCD treatments addressing the solubility of CO_2 in different substrates, effects of pressure, temperature and in combination with exposure time and CO_2 levels. This offers an advantage in thermodynamically predicting solubility of CO_2 in various food components (solids or liquids) at required process conditions most accurately. Thus, this method can profoundly reduce the number of experiments required to establish a standard process protocol for each food substrate in compliance with the regulations.

The final concern in the use of DPCD in food processing at a larger scale is the environmental concern for the disposal of used CO_2. CO_2 being a greenhouse gas poses a threat to the environment and can cause global warming. Apart from the larger picture, CO_2 in the super-critical state under pressure can be a potential hazard in the industry level too to the personnel handling it. It can cause suffocation and restless when inhaled in the purest form. This problem can be combatted by developing a system to trap the used CO_2, purify and regenerate it to form DPCD again and recirculated into the system to create a cycle. This results in very fewer emissions of spent CO_2 into the atmosphere and improves the efficiency and economy of the process.

7. SUMMARY

Dense phase carbon dioxide preservation is a novel non-thermal food preservation technique which utilizes pressurized carbon dioxide to inactivate the microbial and enzymatic activities. This method of food preservation is generally used for heat-sensitive food products especially liquid foods like fruit juices, squashes *etc*., to overcome the losses of nutrients like ascorbic acid, minerals and to nurture the organoleptic properties, which gets quickly deteriorated during thermal processing. Apart from liquid foods, DPCD can also be done to solid foods like in the processing of meat products, fruits and vegetables and other plant matters. To enhance the processing efficiency of DPCD, it can be combined with other non-thermal processing techniques like high pressure processing, pulsed electric field, ultrasound at milder processing conditions for effective microbial reduction and enzyme inactivation which further helps in yielding superior quality food products with reduced or no loss in sensory properties. There are certain advantages and limitations of DPCD in the processing of solid foods. The benefits of DPCD are, it can be operated at mild processing temperatures of 30-50°C without giving up the nutrients like anthocyanin and polyphenols, requires only $1/10^{th}$ of the pressure compared to HHP and automation of carbon di oxide can be done if needed. Thus, DPCD can also be termed as “cold pasteurization”. Though DPCD is a continuous and novel technique, there are few drawbacks and limitations like pure carbon dioxide gas is quite expensive and its usage in large scale industries will not be economical and increases the processing costs heavily. Further research can be done by replacing the CO_2 gas with other gases to make the process cost-effective. DPCD is not used in commercial scale; more work has to be done in the field of preserving solid foods through DPCD. This can conclude that DPCD is an effective and highly feasible technique for

improving the shelf-life and storage stability of liquid and solid foods, along with other applications like DPCD aided extraction of essential oils and high-economic value components from several food ingredients. It is a promising chemical-free technology that helps in food processing without elevating the food temperatures, thus safeguarding the aesthetic and sensory properties of foods acceptable by the end-users.

KEYWORDS:

Carbon dioxide
DPCD
Enzyme
Inactivation
Microbial
Supercritical

8. REFERENCES

1. Arreola, A.G., Balaban, M.O., Marshall, M.R., Peplow, A.J., Wei, C.I. and Cornell, J.A., 1991, Supercritical carbon dioxide effects on some quality attributes of single strength orange juice. *Journal of Food Science*, 56(4): 1030-1033.

2. Bae, Y.Y., Lee, H.J., Kim, S.A. and Rhee, M.S., 2009, Inactivation of Alicyclobacillus acidoterrestris spores in apple juice by supercritical carbon dioxide. *International Journal of Food Microbiology*, 136(1): 95-100.

3. Balaban, M.O. and Duong, T., 2014, Dense phase carbon dioxide research: current focus and directions. *Agriculture and Agricultural Science Procedia*, 2(1): 2-9.

4. Balaban, M.O., Kincal, D., Hill, S., Marshall, M.R. and Wildasin, R., 2001 June, The synergistic use of carbon dioxide and pressure in non-thermal processing of juices. In *IFT annual meeting book of abstracts* (6-3).

5. Ballestra, P., 2012, Effects of dense phase carbon dioxide on bacterial and fungal spores. *Dense Phase Carbon Dioxide: Food and Pharmaceutical Applications*, 99-112.

6. Ballestra, P. and Cuq, J.L., 1998, Influence of pressurized carbon dioxide on the thermal inactivation of bacterial and fungal spores. *LWT-Food Science and Technology*, 31(1): 84-88.

7. Bi, X., Wu, J., Zhang, Y., Xu, Z. and Liao, X., 2011, High pressure carbon dioxide treatment for fresh-cut carrot slices. *Innovative Food Science & Emerging Technologies*, 12(3): 298-304.

8. Calvo, L., Muguerza, B. and Cienfuegos-Jovellanos, E., 2007, Microbial inactivation and butter extraction in a cocoa derivative using high pressure CO_2. *The Journal of supercritical fluids*, 42(1): 80-87.

9. Calvo, L. and Torres, E., 2010, Microbial inactivation of paprika using high-pressure CO2. *The Journal of Supercritical Fluids*, 52(1): 134-141.

10. Cappelletti, M., Ferrentino, G. and Spilimbergo, S., 2015, High pressure carbon dioxide on pork raw meat: Inactivation of mesophilic bacteria and effects on colour properties. *Journal of Food Engineering*, 156(1): 55-58.

11. Casas, J., Tello, J., Gatto, F. and Calvo, L., 2016, Microbial inactivation of paprika using oregano essential oil combined with high-pressure CO_2. *The Journal of Supercritical Fluids*, 116(1): 57-61.

12. Ceni, G., Silva, M.F., Valério Jr, C., Cansian, R.L., Oliveira, J.V., Dalla Rosa, C. and Mazutti, M.A., 2016, Continuous inactivation of alkaline phosphatase and Escherichia coli in milk using compressed carbon dioxide as inactivating agent. *Journal of CO_2 Utilization*, 13(1): 24-28.

13. Chao, R.R., Mulvaney, S.J., Bailey, M.E. and Fernando, L.N., 1991, Supercritical CO2 conditions affecting extraction of lipid and cholesterol from ground beef. *Journal of Food Science*, 56(1): 183-187.

14. Chen, J., Zhang, J., Feng, Z., Song, L., Wu, J. and Hu, X., 2009, Influence of thermal and dense-phase carbon dioxide pasteurization on physicochemical properties and flavor compounds in Hami melon juice. *Journal of agricultural and food chemistry*, 57(13): 5805-5808.

15. Chen, J.S., Balaban, M.O., Wei, C.I., Marshall, M.R. and Hsu, W.Y., 1992, Inactivation of polyphenol oxidase by high-pressure carbon dioxide. *Journal of Agricultural and Food Chemistry*, 40(12): 2345-2349.

16. Chitra, J., Deb, S. and Mishra, H.N., 2015, Selective fractionation of cholesterol from whole milk powder: optimisation of supercritical process conditions. *International Journal of Food Science & Technology*, 50(11): 2467-2474.

17. Dagan, G.F. and Balaban, M.O., 2006, Pasteurization of beer by a continuous dense-phase CO2 system. *Journal of food science*, 71(3): E164-E169.

18. Damar, S. and Balaban, M.O., 2006, Review of dense phase CO_2 technology: microbial and enzyme inactivation, and effects on food quality. *Journal of food science*, 71(1): R1-R11.

19. Damar, S., Balaban, M.O. and Sims, C.A., 2009, Continuous dense-phase CO_2 processing of a coconut water beverage. *International journal of food science & technology*, 44(4): 666-673.

20. Debs-Louka, E., Louka, N., Abraham, G., Chabot, V. and Allaf, K., 1999, Effect of compressed carbon dioxide on microbial cell viability. *Applied and environmental microbiology*, 65(2): 626-631.

21. Dehghani, F., Annabi, N., Titus, M., Valtchev, P. and Tumilar, A., 2009, Sterilization of ginseng using a high pressure CO_2 at moderate temperatures. *Biotechnology and Bioengineering*, 102(2): 569-576.

22. Duong, T., Balaban, M., Perera, C. and Bi, X., 2015, Microbial and sensory effects of combined high hydrostatic pressure and dense phase carbon dioxide process on feijoa puree. *Journal of Food Science*, 80(11): E2478-E2485.

23. Nakamura, K., Enomoto, A., Fukushima, H., Nagai, K. and Hakoda, M., 1994, Disruption of microbial cells by the flash discharge of high-pressure carbon dioxide. *Bioscience, biotechnology, and biochemistry*, 58(7): 1297-1301.

24. Erkmen, O., 2012, Effects of dense phase carbon dioxide on vegetative cells. *Dense phase carbon dioxide: food and pharmaceutical applications*, 67-97.

25. Ferrentino, G., Barletta, D., Donsì, F., Ferrari, G. and Poletto, M., 2010, Experimental measurements and thermodynamic modeling of CO2 solubility at high pressure in model apple juices. *Industrial & Engineering Chemistry Research*, 49(6): 2992-3000.

26. Ferrentino, G., Plaza, M.L., Ramirez-Rodrigues, M., Ferrari, G. and Balaban, M.O., 2009, Effects of dense phase carbon dioxide pasteurization on the physical and quality attributes of a red grapefruit juice. *Journal of Food Science*, 74(6): E333-E341.

27. Folkes, G., 2004, *Pasteurization of beer by a continuous dense-phase carbon dioxide system*. University of Florida.

28. Foster, J.W., Cowan, R.M. and Maag, T.A., 1962, Rupture of bacteria by explosive decompression. *Journal of bacteriology*, 83(2): 330-334.

29. Furukawa, S., Watanabe, T., Tai, T., Hirata, J., Narisawa, N., Kawarai, T., Ogihara, H. and Yamasaki, M., 2004, Effect of high pressure gaseous carbon dioxide on the germination of bacterial spores. *International journal of food microbiology*, 91(2): 209-213.

30. Gangola, P. and Rosen, B.P., 1987, Maintenance of intracellular calcium in Escherichia coli. *Journal of Biological Chemistry*, 262(26): 12570-12574.

31. Garcia-Gonzalez, L., Geeraerd, A.H., Elst, K., Van Ginneken, L., Van Impe, J.F. and Devlieghere, F., 2009, Influence of type of microorganism, food ingredients and food properties on high-pressure carbon dioxide inactivation of microorganisms. *International Journal of Food Microbiology*, 129(3): 253-263.

32. Garcia-Gonzalez, L., Geeraerd, A.H., Spilimbergo, S., Elst, K., Van Ginneken, L., Debevere, J., Van Impe, J.F. and Devlieghere, F., 2007, High pressure carbon dioxide inactivation of microorganisms in foods: the past, the present and the future. *International journal of food microbiology*, 117(1): 1-28.

33. Gui, F., Wu, J., Chen, F., Liao, X., Hu, X., Zhang, Z. and Wang, Z., 2006, Change of polyphenol oxidase activity, color, and browning degree during storage of cloudy apple juice treated by supercritical carbon dioxide. *European Food Research and Technology*, 223(1): 427-432.

34. Guo, M., Liu, S., Ismail, M., Farid, M.M., Ji, H., Mao, W., Gao, J. and Li, C., 2017, Changes in the myosin secondary structure and shrimp surimi gel strength induced by dense phase carbon dioxide. *Food Chemistry*, 227(1): 219-226.

35. Hendrickx, M., Ludikhuyze, L., Van den Broeck, I. and Weemaes, C., 1998, Effects of high pressure on enzymes related to food quality. *Trends in Food Science & Technology*, 9(5): 197-203.

36. Ho-mu, L., Zhiying, Y. and Li, F.C., 1993, Inactivation of Leuconostoc dextranicum with carbon dioxide under pressure. *The Chemical Engineering Journal*, 52(1): B29-B34.

37. Hong, S.I. and Pyun, Y.R., 1999, Inactivation kinetics of Lactobacillus plantarum by high pressure carbon dioxide. *Journal of Food Science*, 64(4): 728-733.

38. Hong, S.I. and Pyun, Y.R., 2001, Membrane damage and enzyme inactivation of Lactobacillus plantarum by high pressure CO_2 treatment. *International Journal of Food Microbiology*, 63(1-2): 19-28.

39. Isenschmid, A., Marison, I.W. and Von Stockar, U., 1995, The influence of pressure and temperature of compressed CO_2 on the survival of yeast cells. *Journal of Biotechnology*, 39(3): 229-237.

40. Jan, A., Sood, M., Sofi, S.A. and Norzom, T., 2017, Non-thermal processing in food applications: A review. *International Journal of Food Science and Nutrition*, 2(6): 171-180.

41. Jones, R.P. and Greenfield, P.F., 1982, Effect of carbon dioxide on yeast growth and fermentation. *Enzyme and Microbial Technology*, 4(4): 210-223.

42. Kim, H.T., Choi, H.J. and Kim, K.H., 2009, Flow cytometric analysis of Salmonella enterica serotype Typhimurium inactivated with supercritical carbon dioxide. *Journal of microbiological methods*, 78(2): 155-160.

43. Kincal, D., Hill, W.S., Balaban, M.O., Portier, K.M., Wei, C.I. and Marshall, M.R., 2005, A continuous high pressure carbon dioxide system for microbial reduction in orange juice. *Journal of Food Science*, 70(5): M249-M254.

44. Lim, S., Yagiz, Y. and Balaban, M.O., 2006, Continuous high pressure carbon dioxide processing of mandarin juice. *Food Science and Biotechnology*, 15(1): 13-18.

45. Li, S., Shao, Q., Lu, Z., Duan, C., Yi, H. and Su, L., 2018, Rapid determination of crocins in saffron by near-infrared spectroscopy combined with chemometric techniques. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 190(1): 283-289.

46. Lim, S., Yagiz, Y. and Balaban, M.O., 2006, Continuous high pressure carbon dioxide processing of mandarin juice. *Food Science and Biotechnology*, 15(1): 13-18.

47. Mantoan, D. and Spilimbergo, S., 2010, Mathematical modeling of yeast inactivation of freshly squeezed apple juice under high-pressure carbon dioxide. *Critical reviews in food science and nutrition*, 51(1): 91-97.

48. Meujo, D.A., Kevin, D.A., Peng, J., Bowling, J.J., Liu, J. and Hamann, M.T., 2010, Reducing oyster-associated bacteria levels using supercritical fluid CO2 as an agent of warm pasteurization. *International Journal of Food Microbiology*, 138(1-2): 63-70.

49. Cappelletti, M., Ferrentino, G. and Spilimbergo, S., 2014, Supercritical carbon dioxide combined with high power ultrasound: An effective method for the pasteurization of coconut water. *The Journal of Supercritical Fluids*, 92(1): 257-263.

50. Cappelletti, M., Ferrentino, G. and Spilimbergo, S., 2014, Supercritical carbon dioxide combined with high power ultrasound: An effective method for the pasteurization of coconut water. *The Journal of Supercritical Fluids*, 92(1): 257-263.

51. Niu, S., Xu, Z., Fang, Y., Zhang, L., Yang, Y., Liao, X. and Hu, X., 2010, Comparative study on cloudy apple juice qualities from apple slices treated by high pressure carbon dioxide and mild heat. *Innovative Food Science & Emerging Technologies*, 11(1): 91-97.

52. Niu, S., Xu, Z., Fang, Y., Zhang, L., Yang, Y., Liao, X. and Hu, X., 2010, Comparative study on cloudy apple juice qualities from apple slices treated by high pressure carbon dioxide and mild heat. *Innovative Food Science & Emerging Technologies*, 11(1): 91-97.

53. Ortuño, C., Duong, T., Balaban, M. and Benedito, J., 2013, Combined high hydrostatic pressure and carbon dioxide inactivation of pectin methylesterase, polyphenol oxidase and peroxidase in feijoa puree. *The Journal of Supercritical Fluids*, 82(1): 56-62.

54. Park, S.J., Lee, J.I. and Park, J., 2002, Effects of a combined process of high-pressure carbon dioxide and high hydrostatic pressure on the quality of carrot juice. *Journal of Food Science*, 67(5): 1827-1834.

55. Pataro, G., Ferrentino, G., Ricciardi, C. and Ferrari, G., 2010, Pulsed electric fields assisted microbial inactivation of S. cerevisiae cells by high pressure carbon dioxide. *The Journal of Supercritical Fluids*, 54(1): 120-128.

56. Patil, P.D., Dandamudi, K.P.R., Wang, J., Deng, Q. and Deng, S., 2018, Extraction of bio-oils from algae with supercritical carbon dioxide and co-solvents. *The Journal of Supercritical Fluids*, 135(1): 60-68.

57. Del Pozo-Insfran, D., Balaban, M.O. and Talcott, S.T., 2006, Microbial stability, phytochemical retention, and organoleptic attributes of dense phase CO_2 processed muscadine grape juice. *Journal of Agricultural and Food Chemistry*, 54(15): 5468-5473.

58. Ramirez-Rodrigues, M.M., Plaza, M.L., Ferrentino, G., Balaban, M.O., REYES-DE CORCUERA, J.I. and Marshall, M.R., 2013, Effect of dense phase carbon dioxide processing on microbial stability and physicochemical attributes of Hibiscus sabdariffa beverage. *Journal of Food Process Engineering*, 36(1): 125-133.

59. Rao, W., Li, X., Wang, Z., Yang, Y., Qu, Y., Gao, Y., Chen, L. and Zhang, D., 2017, Dense phase carbon dioxide combined with mild heating induced myosin denaturation, texture improvement and gel properties of sausage. *Journal of Food Process Engineering*, 40(2): e12404.

60. Rawson, A., Tiwari, B.K., Brunton, N., Brennan, C., Cullen, P.J. and O'donnell, C.P., 2012, Application of supercritical carbon dioxide to fruit and vegetables: Extraction, processing, and preservation. *Food Reviews International*, 28(3): 253-276.

61. Shimoda, M., Cocunubo-Castellanos, J., Kago, H., Miyake, M., Osajima, Y. and Hayakawa, I., 2001, The influence of dissolved CO_2 concentration on the death kinetics of Saccharomyces cerevisiae. *Journal of Applied Microbiology*, 91(2): 306-311.

62. Sirisee, U., Hsieh, F. and Huff, H.E., 1998, Microbial safety of supercritical carbon dioxide processes 1. *Journal of Food Processing and Preservation*, 22(5): 387-403.

63. Spilimbergo, S. and Bertucco, A., 2003, Non-thermal bacterial inactivation with dense CO2. *Biotechnology and Bioengineering*, 84(6): 627-638.

64. Spilimbergo, S., Bertucco, A., Basso, G. and Bertoloni, G., 2005, Determination of extracellular and intracellular pH of Bacillus subtilis suspension under CO_2 treatment. *Biotechnology and bioengineering*, 92(4): 447-451.

65. Spilimbergo, S., Cappelletti, M., Tamburini, S., Ferrentino, G. and Foladori, P., 2014, Partial permeabilisation and depolarization of Salmonella enterica Typhimurium cells after treatment with pulsed electric fields and high pressure carbon dioxide. *Process Biochemistry*, 49(12): 2055-2062.

66. Spilimbergo, S., Dehghani, F., Bertucco, A. and Foster, N.R., 2003, Inactivation of bacteria and spores by pulse electric field and high pressure CO_2 at low temperature. *Biotechnology and bioengineering*, 82(1): 118-125.

67. Spilimbergo, S., Elvassore, N. and Bertucco, A., 2002, Microbial inactivation by high-pressure. *The Journal of Supercritical Fluids*, 22(1): 55-63.

68. Ishikawa, H., Shimoda, M., Tamaya, K., Yonekura, A., Kawano, T. and Osajima, Y., 1997, Inactivation of Bacillus spores by the supercritical carbon dioxide micro-bubble method. *Bioscience, biotechnology, and biochemistry*, 61(6): 1022-1023.

69. Timko, M.T., Marre, S. and Maag, A.R., 2016, Formation and characterization of emulsions consisting of dense carbon dioxide and water: ultrasound. *The Journal of Supercritical Fluids*, 109(1): 51-60.

70. Valley G., Rettger L.F., 1927, The Influence of Carbon Dioxide on Bacteria. *Journal of bacteriology*, 14(2): 101–10137.

71. Valverde, M.T., Marín-Iniesta, F. and Calvo, L., 2010, Inactivation of Saccharomyces cerevisiae in conference pear with high pressure carbon dioxide and effects on pear quality. *Journal of Food Engineering*, 98(4): 421-428.

72. Watanabe, T., Furukawa, S., Tai, T., Hirata, J., Narisawa, N., Ogihara, H. and Yamasak, M., 2003, High pressure carbon dioxide decreases the heat tolerance of the bacterial spores. *Food science and technology research*, 9(4): 342-344.

73. Wei, C.I., Balaban, M.O., Fernando, S.Y. and Peplow, A.J., 1991, Bacterial effect of high pressure CO_2 treatment on foods spiked with Listeria or Salmonella. *Journal of Food Protection*, 54(3): 189-193.

74. Werner, B.G. and Hotchkiss, J.H., 2006, Continuous flow non-thermal CO_2 processing: the lethal effects of subcritical and supercritical CO_2 on total microbial populations and bacterial spores in raw milk. *Journal of Dairy Science*, 89(3): 872-881.

75. Yagiz, Y., Lim, S.L. and Balaban, M.O., 2005, Continuous high pressure CO_2 processing of mandarin juice. In *IFT Annual meeting book of abstracts*, 15-20. Chicago, IL, USA: Institute of Food Technologists.

76. Zhong, Q., Black, D.G., Davidson, P.M. and Golden, D.A., 2008, Non-thermal inactivation of Escherichia coli K-12 on spinach leaves, using dense phase carbon dioxide. *Journal of food protection*, 71(5): 1015-1017.