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Cold Pasteurisation of Liquid Foods using Dense Phase Carbon Dioxide

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Review Article

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ABSTRACT

Liquid foods are generally processed by thermal pasteurization techniques, which can destroy heat-sensitive nutrients and sensory attributes. Dense phase carbon dioxide (DPCD) is a non-thermal technology which can inactivate certain microorganisms in liquid foods at low temperatures to avoid the thermal effects of traditional pasteurization. This technology has been investigated over the past 50 years, particularly in the past 2 decades, it has been proposed as a cold pasteurization technique for liquid foods. This paper is a review of published knowledge concerning the DPCD technique for microbial inactivation, the mechanism of DPCD bactericidal action, experimental and commercial DPCD treatment systems. In addition, the review also reflects on the future scope and especially, the current challenges in the DPCD technique for the food industry.

INTRODUCTION

Liquid food products are pasteurised in order to eliminate the risk of food poisoning and increase their shelf life. Pasteurization is a process in which the liquid foods are heated to a specific temperature for a specific time to kill or deactivate the pathogenic bacteria. The liquid foods that are commonly pasteurized are whole liquid eggs (removed from shell), milk, fruit juices, almonds, cider, and beer.

Usually liquid foods are pasteurized by a low temperature long time (LTLT) process at about 145°F (63°C) for 30 min or a high temperature short time (HTST) process at about 162°F (72°C) for 15 s or an ultra-high temperature (UHT) process at about 265 to 295°F (130 to 145°C) for 2 to 45 s. Though the heat treatment can increase the shelf life to about 2 to 3 weeks under refrigeration (lower than 7°C), it can cause significant reduction in physical, nutritive, sensory quality of foods and may also reduce the content or bioavailability of some bioactive compounds [1-3]. Therefore, there is a necessity for pasteurization using non-thermal techniques such as high pressure processing, irradiation, pulsed electric fields, power ultrasound, ozone, oscillating magnetic fields etc.

The use of dense phase carbon dioxide (DPCD) has been proposed as an alternative non-thermal technique for pasteurization of foods [4]. In the DPCD technique, food is contacted with (pressurized) sub- or supercritical CO_2 for a certain amount of time in batch, semi batch, or continuous equipment. Though DPCD technique has been studied on a variety of food products, it has predominantly been used for pasteurising liquid foods. Various studies have shown that DPCD can be used an effective means to inactivate microorganisms in liquid foods. Increasing research interests in this technology have aimed at developing not only safe foods but also high-quality food with “fresh-like” characteristics.

This paper aims to provide a detailed and critical review of the application of the DPCD technique for pasteurisation of liquid foods, and also to shed some light on its current challenges and opportunities for future development in the food industry.

Dense Phase Carbon Dioxide

Dense phase is the fourth phase (Solid, Liquid, Gas, and Dense) that cannot be described by the senses. When a pure compound is above critical pressure and critical temperature, the system is often referred to as a “dense phase fluid” or “super critical fluid” to distinguish it from normal gas and liquid. The term “dense phase” (DP) fluid as used here denotes those phases

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of matter that remain fluid, yet are dense. The dense phase has a viscosity similar to that of a gas, but a density closer to that of a liquid. Because of its unique properties, dense phase has become attractive for food and pharmaceutical processing applications, transportation of natural gas, carbon dioxide (CO_2).

CO_2 can be more beneficially utilised in its dense form for pasteurisation of liquid foods due to its demonstrated bactericidal effects [5-7]. While other gasses (N_2O, N_2, Ar, tetrafluoroethane) have also been evaluated for bactericidal efficacy at pressures higher than atmospheric, the superior effect that CO_2 has over other gases has been attributed to its low critical point: 7.11 MPa, 31°C, which is only slightly above room temperature, thereby eliminating thermal degradation **(Table 1)**.

Table 1. Properties of some supercritical fluids at the critical point [8].

Fluid	Critical Temp. (°C)	Critical Pressure (MPa)	Critical Density (g/ml)
CO_2	31.0	7.11	0.47
N_2O	36.5	7.10	0.45
Water	374.2	21.50	0.32

Moreover, in the dense/supercritical phase, CO_2 has low viscosity ($3–7 \times 10^{-5}$ Nsm^{-2}) and zero surface tension, so it can quickly penetrate porous and complex food materials. CO_2 remains the most preferable gas for killing food organisms because of its low toxicity, nonflamability, and low cost [9] making it an economically feasible option. Furthermore, as an additive, CO_2 would not negatively affect a consumer's perception because of their familiarity with products such as carbonated beverages.

Earlier Uses of Carbon Dioxide in Food Preservation

Because of the need for a preservation method that is safe, inexpensive, and non-destructive to heat sensitive compounds, the use of carbon dioxide has been tested as a food preservation method. The use of carbonation as a means of preserving food started as early as 1939 with the study by Brown et al. [10] where apple cider was carbonated and microbial inactivation and flavor changes were recorded. The carbonation of the juice was shown to preserve the cider for up to 3 months at approximately 21°C with no change in flavor.

The use of carbonation was also investigated for its use in soft drinks as a preservation agent. Even at the lowest amount of gas pressure (3 volumes of CO_2 where 1 volume=1 L of CO_2 per L of beer) sterility was achieved on approximately the 20th day depending on the °Brix of the beverage [11]. Further, since 1980, many researchers have reported the bacteriostatic action and inhibitory effect of CO_2 on growth and metabolism of some microorganisms. Although carbonation with CO_2 has been shown as an effective preservative some bacteria are not affected.

Molin [12] reported that CO_2 had approximately 75% inhibitory affect on *Bacillus cereus*, *Brochothrix thermosphacta*, and *Aeromonas hydrophila*, and a 53% - 29% inhibitory effect on *Escherichia coli* and *Streptococcus faecalis*. *Pseudomonas* was found to be very sensitive while other types, such as *Lactobacillus* and *Clostridium* were less sensitive. Inhibitory rates for anaerobic bacteria were even lower. This proved that carbonation of foods alone would not inactivate all food related bacteria and subsequently brought in the need to use CO_2 under pressure.

Kamihira et al. [13] tested the sterilizing effect of CO_2 at supercritical, liquid and gaseous states on wet and dry *Escherichia coli*, *Staphylococcus aureus* and conidia of *Aspergillus niger* by using a supercritical fluid extraction apparatus. Since then, many studies investigated the effects of CO_2 under pressure on pathogenic and spoilage organisms, vegetative cells and spores, yeasts and molds, enzymes and their activities and food quality attributes. Eventually, it has been found that if CO_2 is pressurized, the process is no longer bacteriostatic, but bactericidal [7,14,15]. Hence, like any technique, the use of CO_2 under pressure to kill bacteria has also been tested on a wide range of food materials using different pressure and temperature conditions.

Furthermore, according to the pressure and temperature conditions used, the application of CO_2 under pressure is denoted by a few techniques. As the terminologies are interchangeably used, it might be necessary to learn the differences between these terminologies to avoid misconceptions.

- HPCD (High Pressure CO_2) Processing - use of CO_2 above atmospheric pressure
- SCCD (Super Critical CO_2) Processing - use of CO_2 in the Super Critical phase only
- DPCD (Dense Phase CO_2) Processing - use of CO_2 in the Super Critical and liquid states

Dense Phase Carbon Dioxide Processing

Dense phase carbon dioxide (DPCD) processing is a non-thermal processing technology that utilizes carbon dioxide at pressures under 50 MPa to inactivate microorganisms i.e., pasteurise mostly liquid foods. DPCD is called as a cold pasteurization method for since it affects microorganisms through molecular effects of CO_2 without exposing foods to adverse thermal effects of traditional pasteurization (heat) and retains their fresh-like physical, nutritional, and sensory qualities [16]. DPCD processing is a collective term for liquid CO_2 and supercritical CO_2 or high pressure carbon dioxide (HPCD).

Fraser [17] was the first to show that DPCD can inactivate bacterial cells. He showed that 99% of *E. coli* numbers were rendered non-viable by a decompression of CO_2 from 500 psi to atmospheric pressure. Since then, many studies investigated the effects of DPCD on pathogenic organisms and enzymes. Works on various liquid foods have been carried on by many investigators **(Table 2)**. In the past 2 decades, the number of research results and patents increased, and commercialization efforts intensified [18].

Table 2. Summary of the studies on microbial inactivation in liquid foods by dense phase CO_2.

S. No.	Food	Inoculated Microorganism	Treatment	Temperature (°C)	System	Maximum Reduction	Reference
1	Milk	*L. monocytogenes*	7 MPa /1 h	45	Batch	3 log	Lin et al. [19]
2	Skim milk	*S. aureus*	9-15 MPa /2-5 h	25	Batch	7 log	Erkmen [20]
3	Fruit juice	*E. faecalis*	6 MPa/3-6 h	45	Batch	5 log	Erkmen [21]
4	Milk	*E. faecalis*	6 MPa/24 h	45	Batch	5 log	Erkmen [21]
5	Orange juice	*L. monocytogenes*	6 MPa/8 h	45	Batch	6 log	Erkmen [22]
6	Peach juice	*L. monocytogenes*	6 MPa/4 h	45	Batch	6 log	Erkmen [22]
7	Whole milk	*E. coli*	10 MPa/6 h	30	Batch	6.4 log	Erkmen [15]
8	Skim milk	*E. coli*	10 MPa/6 h	30	Batch	7.2 log	Erkmen [15]
9	Natural orange juice	*Mould-Yeasts*	30 MPa/15 min	28	Semi-continuous	Total inactivation	Spilimbergo et al. [4]
10	Beer	Yeasts	26.5 MPa/4.77 min 9.6% CO_2	21	Continuous	7.3 log	Folkes [23]
11	Grape juice	*S. cerevisiae*	49 MPa 170 g CO_2/kg juice	25	Continuous	5.5 log	Gunes et al. [24]
12	Orange juice	*S. typhimurium*	21 MPa/10 min	25	Continuous	6 log	Kincal et al. [25]
13	Orange juice	*L. monocytogenes*	38 MPa/10 min	25	Continuous	6 log	Kincal et al. [25]
14	Coconut water	Aerobic plate count	34.5 MPa/6 min 13% CO_2	40	Continuous	5.61log	Damar [26]
15	Apple juice	*S. cerevisiae*	9 MPa/15 min	32	Multi-batch	4. 9 log	Parton et al. [27]
16	Kava beverage	Aerobic plate count	34.5 MPa/7 min	30	Continuous	3 log	Hsieh et al. [28]
17	Apple juice	Aerobic plate count	10 MPa/10 min	36	Multi-batch	Total inactivation	Gasperi et al. [29]
18	Hami melon juice	Aerobic plate count	35 MPa/60 min	55	Batch	Total inactivation	Zhang et al. [30]
19	Peach and kiwi juice	*S. cerevisiae*/Aerobic plate count	10 MPa/15 min	35	Batch	Total inactivation	Spilimbergo and Ciola [31]
20	Lychee juice	Aerobic plate count	8 MPa/2 min	36	Batch	5 log	Guo et al. [32]

Mechanisms of Microbial Inactivation by Dpcd

Though many theories were developed to explain the bacteriostatic action of CO_2, the exact inactivation mechanisms still remain to be unravelled. However, the different steps involved in the hypothetical microbial inactivation mechanism can be summarized as follows:

i) Solubilization of pressurized CO_2 in the external liquid phase

ii) Cell membrane modification

iii) Intracellular pH lowering effect

iv) Key enzyme inactivation

v) Direct (inhibitory) effect of molecular CO_2 and HCO_3 on metabolism

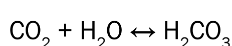
vi) Disordering of the intracellular electrolyte balance

vii) Removal of vital constituents from cells and cell membranes.

Most of these steps will not occur consecutively, but rather take place simultaneously in a complex and interrelated manner.

Solubilization of pressurized Co_2 in the external liquid phase

Pressurized CO_2 is able to dissolve in the water content of the food matrix. As a consequence, the aqueous part of a food in contact with pressurized CO_2 generally becomes acidic due to the formation and dissociation of H_2CO_3, which liberates H^+ ions.



$$CO_2 + H_2O \leftrightarrow H_2CO_3$$

$$H_2CO_3 \leftrightarrow H^+ + HCO_3^- \qquad pKa=6.57$$

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$HCO_3 \leftrightarrow H^+ + CO_3^{-2}$ pKa=10.62

This lowered extracellular pH may diminish microbial resistance to inactivation because of the increased energy consumption to maintain pH homeostasis. CO_2 shows an inhibitory effect greater than that of the other acids and penetrate cells at a much faster rate than other molecules which do not produce acidification in solution. The lower external pH contributes to an increase in cell permeability and facilitates the penetration of CO_2 into microbial cells at higher rate [19,33].

Cell membrane modification

The aqueous CO_2 approaches the surface of the bacterial cells, diffuse into the cellular membrane and may accumulate in its lipophilic (phospholipid) inner layer. Generally, there is high affinity between CO_2 and the plasma membrane. CO_2 can be dissolved in the phospholipids of a model cell membrane, principally phosphotidietanol amines and phosphotiliglycerol at a very high extent. 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") which may increase the fluidity, and then the permeability of the membrane [4].

Intracellular ph lowering effect

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 dissolved CO_2 and HCO_3^- 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 internal pH (which is essential for optimal cell viability and cellular activity). However, if too much dissolved CO_2 enters the cytoplasm, the internal pH will start to decrease. If the internal pH is lowered too much, cell viability will be seriously impaired and the cells also may be unable to maintain the resulting large pH difference, ΔpH (ΔpH = pH internal – pH external). Therefore, impairment of cellular activity may coincide with both a low internal pH and the collapse of a large ΔpH [34].

Spilimbergo et al. [8] exposed a suspension of *Bacillus subtilis* to DPCD at 8.0 MPa and 30 °C for 5 min. The internal pH and external pH of the suspension were determined as 3.3 and 3.2 respectively which brought up a 5-decimal reduction of *B. subtilis* cells. Though the values of internal pH and external pH were very similar, these findings seemed to support the hypothesis that upon DPCD exposure, microbial cells are unable to maintain a favourable cytoplasmic pH homeostasis and many aspects of cell structure and function are influenced by internal pH.

Key enzyme inactivation

Enzymes, which make up most of the proteins in the cytosol, have maximal activity at the optimum pH, and their activity declines sharply on either side of the optimum. Lowering of the cytosolic internal pH might cause inhibition and/or inactivation of key enzymes essential for metabolic and regulatory processes, such as glycolysis, amino acid and peptide transport, active transport of ions, and proton translocation [34]. Thus a loss over biological control of the internal pH of cells may be detrimental in all aspects of intermediary metabolism and cellular function.

Direct (inhibitory) effect of molecular Co_2 and Hco_3 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 substrates, products, and cofactors, which are primary elements in the regulation of enzymatic activity.

Carboxylation reactions are particularly important for the gluconeogenesis and the synthesis of particular biosynthetic precursor amino acids and nucleic acids. CO_2 fulfils the role of either a biosynthetic substrate in carboxylation reactions or a metabolic product from decarboxylation reactions. As far as decarboxylation reactions are concerned, they all appear to produce CO_2 in the dissolved (unhydrated) CO_2 form.

The ultimate effect that the various species of CO_2 may have on microbial metabolism probably will be a function of the relative importance of the various carboxylation and decarboxylation reactions [35].

Disordering of the intracellular electrolyte balance

When the applied CO_2 pressure accumulates in the cytoplasmic interior of the bacterial cells, lethal damage to the biological system of the cells may occur. This may convert HCO_3^- to CO_3^{2-}, which could precipitate intracellular inorganic electrolytes (such as Ca^{2+}, Mg^{2+} and similar ions) from cells and cell membranes [33]. Since these inorganic electrolytes (apart from being important regulators of a large number of other cell activities) help in maintaining the osmotic relationships between cells and their surrounding media, this could have deleterious effects on the volume of cells.

Removal of vital constituents from cells and cell membranes

Several authors have suggested that accumulated CO_2 could, due to its relatively high solvating power, "extract" vital constituents from the cells or cell membranes. The pressurized CO_2 first penetrates into the cells to build up the density to a critical level within the cells. Then there is a sudden release of the applied pressure which disturbs or alters the structure of the bio-membrane, leading to removal and rapid transfer of the intracellular constituents, such as phospholipids and hydrophobic compounds out of the biological system into the extracellular environment. This extraction disturbs the balance of the biological

system, thus promoting inactivation [13,33,36]. Lin et al. [36] also suggested that the rate of inactivation could be improved by repeating the release and recharge of pressurized CO_2 in the pressure vessel during the treatment.

Nakamura et al. [7] used SEM as an evidence for mechanical rupture of yeast cells. They observed that some cells were completely burst whereas some had wrinkles or holes on their surface. This was also confirmed by Ballestra et al. [37] who demonstrated that some *E. coli* cells treated with DPCD at 5.0 MPa and 35 °C showed some signs of deformation of cell walls. Following researches by Hong and Pyun [38], Folkes [23] and Bertoloni et al. [39] in the *L. plantarum, E. coli and S. cerevisiae* revealed DPCD treatment resulted in irreversible cellular membrane damage including leakage of intracellular materials like UV absorbing substances, enzymes and release of intracellular ions (such as Mg^{2+} and K^+).

Current Challenges and Future Scope

After a comprehensive review of prior research on Dense phase CO_2 processing method to pasteurise liquid foods, it can be observed that there are some challenges remain.

- Only vegetative bacteria have been extensively studied and are susceptible to Dense Phase CO_2 treatment
- The effects of different factors like temperature, pressure and equipment on deactivation of microbes can be substantial, but there is not yet a clear understanding of these effects
- The nutritional attributes of DPCD treated liquid foods in general have been poorly examined
- The shelf-life and stability of the DPCD treated liquid foods during different storage conditions has not sufficiently been studied

The future research on DPCD treatment for liquid foods may be directed towards the following line of action:

- Mathematical modelling of CO_2 pasteurization need to be strengthened, as it is important for elucidating the mechanisms, and for process optimization
- Combined treatments of dense phase carbon dioxide and other potential non thermal processing techniques like high hydrostatic pressure (HHP) can be studied to enhance the safety and shelf life of liquid foods

CONCLUSION

There has been an increased interest in application of non thermal techniques to preserve liquid foods. At the same time there is also a demand for fresh-like and nutritious food. The DPCD cold pasteurisation technology is a cost-efficient, environment friendly and reliable method to preserve the quality of liquid food. Further, several batch, semi-continuous, and continuous systems have been developed for DPCD applications. However, the applicability of this technology in industrial scale has not been widely spread due to be challenges mentioned above. It is necessary to have a better understanding of the complex challenges and find out the ways that would contribute to improve the applications of DPCD for pasteurization of liquid foods.

REFERENCE

1. Patras A, et al. Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. Trends in Food Science & Technology. 2010;21:3-11.
2. Patras A, et al. Effect of thermal and high pressure processing on antioxidant activity and instrumental colour of tomato and carrot purées. Innov Food Sci Emerg Technol. 2009;10:16-22.
3. Rawson A, et al. Effect of thermal and non thermal processing technologies on the bioactive content of exotic fruits and their products: Review of recent advances. Food Res Int. 2011;44:1875-1887.
4. Spilimbergo S, et al. Microbial inactivation by high pressure. Journal of Supercritical Fluids. 2002;22:55-63.
5. Balaban MO, et al. Inactivation of pectinesterase in orange juice by supercritical carbon dioxide. J Food Sci. 1991;56:743-746.
6. Wei CI, et al. Bacterial effect of high pressure CO_2 treatment on foods spiked with Listeria or Salmonella. J Food Prot. 1991;54:189-193.
7. Nakamura K, et al. Disruption of microbial cells by the flash discharge of high-pressure carbon dioxide. Biosci Biotechnol Biochem 1994;58:1297-1301.
8. Spilimbergo S, et al. Determination of extracellular and intracellular pH of Bacillus subtilis suspension under CO_2 treatment. Biotechnol Bioeng. 2005;92:447-451.
9. Isenschmid A, et al. The influence of pressure and temperature of compressed CO_2 on the survival of yeast cells. J Biotechnol. 1995;39:229-237.
10. Brown HD, et al. Preserving cider by carbonation. Am Soc of Hortic Sci. 1939;36:371-373.

e-ISSN:2321-6204
p-ISSN:2347-2359

11. Insalata N. CO_2 versus beverage bacteria. Food Eng. 1952;190;84-85.

12. Molin G. The resistance to carbon dioxide of some food related bacteria. Eur J Appl Microbiol Biotechnol. 1983;214-217.

13. Kamihira M, et al. Sterilization of microorganisms with supercritical CO_2. Agric Biol Chem. 1987;51:407-412.

14. Arreola AG, et al. Effect of supercritical carbon dioxide on microbial populations in single strength orange juice. J Food Qual. 1991;14:275-284.

15. Erkmen O. Effects of high-pressure carbon dioxide on Escherichia coli in nutrient broth and milk. Int J Food Microbiol. 2001;65:131-135.

16. Dagan GF and Balaban MO. Pasteurization of beer by a continuous dense phase CO_2 system. J Food Sci. 2006;71:164-169.

17. Fraser D. Bursting bacteria by release of gas pressure. Nature. 1951;167:33-34.

18. Jones RP and Greenfield PF. Effect of carbon dioxide on yeast growth and fermentation. Enzyme Microb Technol. 1982;4:210-223.

19. Lin HM, et al. Antimicrobial effect of pressurized carbon dioxide on Listeria monocytogenes. J Food Sci. 1994;59:657-659.

20. Erkmen O. Antimicrobial effect of pressurized carbon dioxide on staphylococcus aureus in broth and milk. LWT-Food Sci Techn. 1997;30:826-829.

21. Erkmen O. Antimicrobial effect of pressurised carbon dioxide on Enterococcus faecalis in physiological saline and foods. J Sci Food Agric. 2000;80:465-470.

22. Erkmen O. Effect of carbon dioxide pressure on Listeria monocytogenes in physiological saline and foods. Food Microbiology. 2000b;17:589-596.

23. Folkes G. Pasteurization of beer by a continuous dense-phase CO_2 system. PhD dissertation, Fl University of Florida, Gainesville. 2004;110.

24. Gunes G, et al. Inactivation of yeasts in grape juice using a continuous dense phase carbon dioxide processing system. J Sci Food Agric. 2005;85:2362-2368.

25. Kincal D, et al. A continuous high pressure carbon dioxide system for microbial reduction in orange juice. J Food Sci. 2005;70:M249-M254.

26. Damar S. Processing of coconut water with high pressure carbon dioxide technology. PhD dissertation, University of Florida. 2006.

27. Parton T, et al. A continuous plant for food preservation by high pressure CO_2. J Food Eng. 2007;79:1410-1417.

28. Hsieh M, et al. The quality improvement of kava beverage by non-thermal dense phase CO_2 pasteurization. Institute of Food Technologists Annual Meeting, Chicago, Illinois. 2007;189-158.

29. Gasperi F, et al. Effects of supercritical CO_2 and N_2O pasteurisation on the quality of fresh apple juice. Food Chem. 2009;115:129-136.

30. Zhang J, et al. Changes in microorganism, enzyme, aroma of Hami melon (Cucumis melo L.) juice treated with dense phase carbon dioxide and stored at 4°C. Innov Food Sci Emerg Technol 2010;11:623-629.

31. Spilimbergo S and Ciola L. Supercritical CO_2 and N_2O pasteurisation of peach and kiwi juice. Int J Food Sci Technol. 2010;45:1619-1625.

32. Guo M, et al. Effects on microbial inactivation and quality attributes in frozen lychee juice treated by supercritical carbon dioxide. Eur Food Res Technol. 2011;232:803-811.

33. Lin HM, et al. Inactivation of Leuconostoc dextranicum with carbon dioxide under pressure. Chemical Engineering Journal. 1993;52:B29-B34.

34. Hutkins RW and Nannen NL. PH homeostasis in lactic-acid bacteria. J Dairy Sci. 1993;76:2354-2365.

35. Rizvi SSH, et al. Supercritical fluid extraction: operating principles and food applications. Food Technology (USA). 1986;40:57-64.

36. Lin HM, et al. Inactivation of Saccharomyces cerevisiae by supercritical and subcritical CO_2. Biotechnol Prog. 1992;8;458-461.

37. Ballestra P, et al. Inactivation of Escherichia coli by carbon dioxide under pressure. J Food Sci. 1996;61:829-831.

38. Hong SI and Pyun YR. Membrane damage and enzyme inactivation of Lactobacillus plantarum by high pressure CO_2 treatment. Int Journal of Food Microbiol. 2001;63:19-28.

39. Bertoloni G, et al. A study on the inactivation of micro-organisms and enzymes by high pressure CO_2. Journal Biotechnol Bioeng. 2006;95:155-160.