Enhancing DPCD in Liquid Products by Mechanical Inactivation Effects: Assessment of Feasibility

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The enhancement of standard dense phase carbon dioxide (DPCD) pasteurization by additional mechanical effects was assessed in this work. These effects were induced during pasteurization by the sudden depressurization in a narrow minitube. The high flow velocities, moderate pressures (40–80 bar) and low temperatures (25–45 °C) lead to intense degasification and shear stress. The inactivation of the test microorganism *Escherichia coli* DH5α (*E. coli* DH5α) was determined before and after depressurization in the minitube, representing entirely chemical DPCD via dissolved CO_2 and total inactivation comprising the effects of dissolved CO_2 and mechanical effects, respectively. Compared to conventional DPCD pasteurization, which is mostly attributed to chemical effects, the additional mechanical effects increased the inactivation efficiency considerably.

Keywords: Dense phase carbon dioxide, Mechanical effects, Non-thermal microbial inactivation, Pasteurization, Shear stress

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1 Introduction

During the last decades, the growing demand for fresh food products steadily encouraged the development of food preservation methods based on non-thermal effects [1], such as high pressure processes [2], mechanical steam decontamination [3, 4] or dense phase carbon dioxide (DPCD) pasteurization [5–8]. In this context, Bevilacqua et al. recently published an extensive review on non-thermal technologies for fruit and vegetable juices and beverages [9]. The principles of chemical inactivation using DPCD have been extensively described in the literature [10, 11]. Chemical inactivation is attributed to the penetration of carbon dioxide into the bacterial cells leading to an acidification of the bacterial cytoplasm. After explosive decompression of the system, the carbon dioxide rapidly expands, leaves the cells and extracts vital cellular components. Furthermore, beneficial effects of DPCD pasteurization on the preservation of flavors and the increase of in vitro lycopene bioaccessibility in tomato juice were reported by Zhao et al. [12, 13]. DPCD pasteurization processes have been developed for liquid food like juices and other beverages but are still not commonly applied in industry [14].

In the present research, the impact of a pressure drop induced by the sudden depressurization and degassing of aqueous media at high flow velocities, moderate temperatures (25–45 °C) and pressures (50–80 bar) in a minitube was assessed. *Escherichia coli* DH5α was chosen as a model organism in this study because it is a well-known contaminant in fresh food products [15].

This new DPCD approach for rapid microbial inactivation offers the possibility to induce an additional and significant mechanical inactivation effect. For this purpose, a liquid saturated with carbon dioxide flows from a high-pressure vessel into a low-pressure vessel through a minitube. Degasification forces the liquid to flow at high velocity in the tube section with reduced diameter, where bubbles form in the minitube and the gas volume fraction strongly increases. It is important to note that compared to conventional DPCD microbial inactivation, the mechanical treatment time in the minitube is very short and varies between 10 and 15 s depending on the starting operating pressure.

In particular the determination of the log reduction factor (LRF) of colony forming units (CFU) per mL of *E. coli* DH5α resulting from chemical DPCD inactivation as well as from total DPCD including mechanical inactivation is

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reported and the data obtained are compared for experiments carried out with different starting inactivation pressures.

2 Materials and Methods

The experimental setup used in this work is depicted in Fig. 1.

- mass flow transmitter (FT) Bronkhorst Mini Cori-flow for fluids and gases with the range 0.3–30 kg h^{-1} for −10 °C to +50 °C. The accuracy is either ±0.2 % reading for liquids or ±0.5 % reading for gases.
- pressure transmitter (PT) UNIK 5000 with the range 0–70 bar for 10 °C to +50 °C, accuracy to ±0.04 % full scale.
- high- and low-pressure inactivation vessels (Parr Instruments, USA) made from stainless steel and equipped with a double jacket (working pressure up to 200 bar and working temperature up to 350 °C).
- minitube (length 230 mm, inner diameter 0.57 mm and cross section 0.255 mm^2).

2.1 Experimental Setup and Inactivation Trials

The vessel temperature was adjusted by means of a double jacket and a heating-refrigerating circulator. Furthermore, an additional heating jacket was wound around a 50-L CO_2 bottle (LCS IsoTherm, Germany) in order to adjust the starting inactivation pressure by operating a dedicated pressure regulator. 50 mL of the liquid to be inactivated (*E. coli* DH5α suspension in LB broth, $OD_{600} \approx 0.8$) were filled the high-pressure inactivation vessel (Fig. 1) and then pressurized with CO_2. During the filling of the high-pressure vessel with CO_2 the "shut off" valve after pressure regulator was opened and all other valves were closed. The end of the minitube was connected with the low-pressure vessel (on the left side, Fig. 1), which was set to release the pressure to the surroundings at atmospheric pressure. After pressure stabilization in the high-pressure vessel (60 min), a sample was taken, the corresponding CFU (colony forming units) per mL, indicating the bacteria concentration, was measured and the resulting chemical DPCD inactivation was calculated. Subsequently, the valve connecting the tube and high-pressure inactivation vessel was rapidly opened.

Through sudden depressurization the saturated liquid was forced from the high-pressure vessel (40 to 80 bar) to the low-pressure vessel (left). Only a few seconds were necessary for the liquid to pass through the minitube. The mass flow was measured by means of a mass flow transmitter and a second sample was taken from the low-pressure vessel. The bacteria concentration in CFU was measured in this sample before as well as after inactivation (DPCD and total inactivation, including mechanical effect induced by flow through the minitube).

2.2 Preparation of the Liquid for Inactivation

E. coli DH5α (Invitrogen™ competent cells, Thermo Fisher Scientific), long term stored as glycerol stocks at −80 °C, were streaked onto LB (Luria-Bertani) agar (Luria/Miller, Carl Roth) plates to obtain isolated colonies after incubation at 37 °C for 24 h. For starting the first small bacterial overnight culture, a single bacterial colony was transferred with a sterile plastic loop to 5 mL LB media (Luria/Miller, Carl Roth) and incubated overnight at 37 °C in a shaking incubator at 150 rpm in sterile plastic bacterial culture tubes. On the next day the bacteria culture was diluted 1:100 in 50–100 mL LB media in an Erlenmeyer flask and incubated overnight at 37 °C and 150 rpm in a shaking incubator. The optical density (*OD*) of the final bacterial suspension was measured and adjusted to $OD_{600} \approx 0.8$.

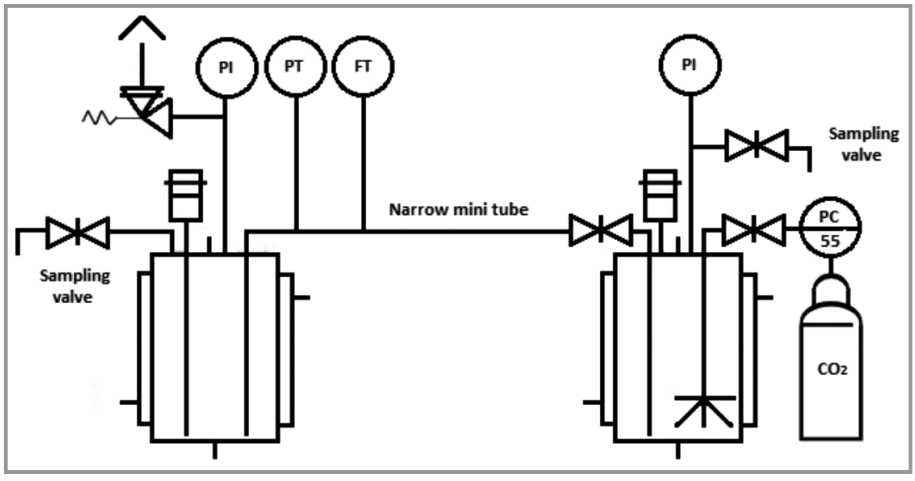

Figure 1. Experimental setup used in this work.

2.3 Bacterial Count

Bacterial culture concentrations were determined by counting the colony forming units on LB agar plates. Samples were serially diluted up to a dilution factor 10^{-8} in sterile PBS (phosphate-buffered saline, Life technologies). 50 µL of 10^{-4} to 10^{-8} dilutions each were deposited to LB agar plates (at least in duplicates). After incubation for 24 h at 37 °C colonies were counted.

3 Results and Discussion

The results of the decontamination trials carried out at three different temperatures (25 °C, 30 °C and 45 °C) by applying different pressure drops under subcritical and supercritical conditions are summarized in Tab. 1 and Fig. 2. The replicates are independent experiments, which were performed under the nominally identical conditions, but not at the same time. While chemical inactivation due to dissolved CO_2 led to a LRF < 0.4, the sudden depressurization and the resulting flow through the minitube led to a significant improvement of the microbial inactivation for all trials. Since both flow velocity and degasification depend on the applied pressure drop, a more efficient inactivation up to LRF = 1.90 (pressure drop = 48.48 bar, temperature = 30 °C) was reached at higher pressure drop. As expected for mechanical effects, the investigated inactivation effect was not strongly dependent on temperature .

Table 1. Results of decontamination trials performed at different temperatures and starting pressures (three replicates each).

Temperature [°C]	Starting pressure [bar]	Pressure drop [bar]	Chemical inactivation – LRF	Total inactivation – LRF
25	56	43.3	0.24	0.45
25	58	44.51	0.26	0.93
25	70	54.13	0.19	1.83
30	62	48.48	0.18	1.90
45	80	65.3	0.03	1.65

These findings indicate that the DPCD decontamination was enhanced by additional mechanical effects. These effects are attributed, among others, to a combination of high flow velocity (high shear stress, acceleration) and degasification (sudden formation of bubbles and increasing gas volume fraction). The average mass flow was (4.37 ± 0.65) g s^{-1}. Compared to the inactivation by both chemical and mechanical means, the exclusive chemical inactivation was not found to be particularly effective and was also not significantly enhanced by applying higher starting inactivation pressures (cf. Fig. 2). A strong increase in total inactivation was observed for pressure drop values above 48 bar, which seems to be a critical value for this process.

4 Conclusions

In this work, DPCD inactivation of *E. Coli* DH5α was shown to be strongly enhanced up to 1.7 log higher than in conventional DPCD pasteurization by inducing additional mechanical effects, which result from the sudden depressurization of the saturated liquid in a minitube. The observed inactivation was attributed to the degasification and the high shear stress resulting from high liquid flow velocities. Compared to conventional DPCD microbial inactivation, the process reported here operates at much lower pressures and temperatures and requires shorter treatment times. Future work will focus on studying the flow and associated

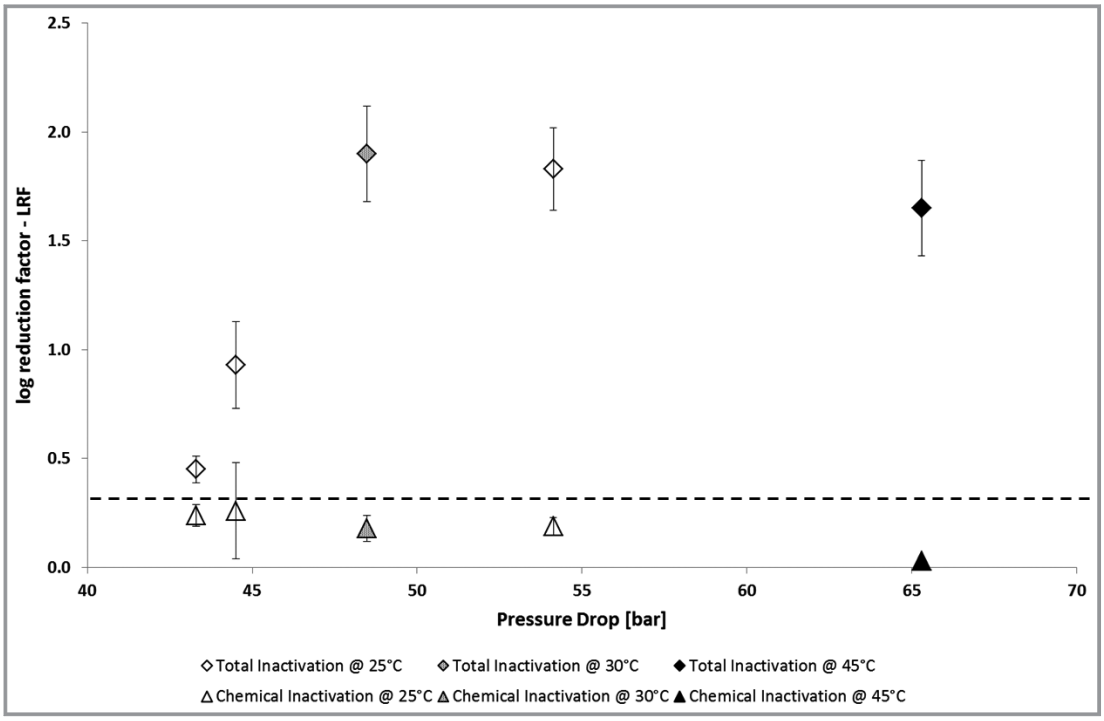

Figure 2. Impact of the pressure drop measured in the minitube on chemical DPCD and total inactivation.

shear forces inside the minitube and on the improvement of LRF. For this purpose, the numerical approach developed by Kneer et al. [16, 17] for the prediction of flow patterns during evaporative carbon dioxide cooling in minitubes will be extended in order to model the flow dynamics involved in this process.

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