Lethality of high-pressure carbon dioxide on Shiga toxin-producing *Escherichia coli, Salmonella* and surrogate organisms on beef jerky

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Abstract:

Low water activity (a_W) foods permit the survival of low-infectious dose pathogens including *Escherichia coli* and *Salmonella*. Desiccation of non-heat resistant *E. coli* and *Salmonella enterica* increases their heat resistance; therefore, a alternative methods are necessary to ensure the safety of low a_W foods. High-pressure carbon dioxide (HPCD) reduced microbial contaminants in high a_W foods. This study aimed to identify HPCD conditions that reduce pathogenic *E. coli* and *Salmonella* in low a_W conditions. Four strains of Shiga toxin-producing *E. coli* (STEC) and one strain of enteropathogenic *E. coli* were treated as a cocktail, and five strains of *Salmonella* were treated individually. The suitability of *E. coli* AW1.7, *Pediococcus acidilactici* FUA 3072, *Enterococcus faecium* NRRL B-2354 and *Staphylococcus carnosus* R6 FUA 2133 as surrogate organisms was evaluated. Treatments were validated in beef jerky. Samples were equilibrated to a_W 0.75 and treated with heat, HPCD or pressurized N_2. Treatment of desiccated *E. coli* AW1.7 and the STEC cocktail with dry gaseous CO_2 (5.7 MPa and 65 °C) did not reduce cell counts; however, treatment with gaseous CO_2 saturated with water reduced cell counts of all strains of *E. coli*. Treatment of beef jerky inoculated with *E. coli* and *Salmonella* with saturated gaseous CO_2 resulted in greater than 5-log reductions for all strains. *E. faecium* NRRL B-2354 and *S. carnosus* R6 were suitable surrogates for *Salmonella* on beef jerky treated with HPCD. Treatment of beef jerky with water-saturated gaseous CO_2 was more effective than treatment with supercritical CO_2 or treatments with N_2 at the same temperature and pressure. Overall, the treatment of low a_W foods with water-saturated gaseous HPCD can meet industry standards by achieving a greater than 5-log reductions of *E. coli* and *Salmonella*. Additionally, surrogate organisms representing pathogenic *E. coli* and *Salmonella* have been validated.

Keywords: enterohaemorrhagic *E. coli*, *Salmonella*, high pressure carbon dioxide, beef jerky, meat safety, verotoxin

1. Introduction

Drying of foods prevents bacterial growth; however, low-infectious dose pathogens may survive at low water activity (a_W) for extended periods of time and cause foodborne illness (Baylis et al., 2004; Gruzdev et al., 2012). *Salmonella enterica* and Shiga toxin-producing *Escherichia coli* (STEC) are estimated to cause 30% of hospitalizations and 24% of deaths from foodborne illness each year in Canada (Health Canada, 2016). The infectious dose of *S. enterica* may be reduced when present in low a_W foods due to the low moisture and high fat content that protects cells from acid conditions during gastric transit (Pan et al., 2012). After desiccation, *E. coli* and *S. enterica* also resist heat treatments that are lethal to hydrated cells (Finn et al., 2013; Li and Gänzle, 2016). Heat resistance is improved by the osmotic stress-induced accumulation of solutes (Calhoun and Frazier, 1966; Finn et al., 2013; Goepfert et al., 1970). This presents a challenge to the food industry because pathogens are frequently controlled by thermal interventions (Nakamura et al., 1994). Therefore, it is imperative to develop novel intervention technologies to reduce *E. coli* and *Salmonella* in low a_W food products.

High-pressure CO_2 (HPCD) inactivates bacterial cells by acidification of the intra- and extra-cellular environment, which disrupts enzyme activity and cellular metabolism (Garcia-Gonzalez et al., 2007). Antimicrobial effects of supercritical and subcritical HPCD in high a_W foods are well documented (Dillow et al., 1999; Garcia-Gonzalez et al., 2007; Lin et al., 1992; Wei et al., 1991). However, acidification is dependent on the presence of water, and only few studies have investigated the effects of HPCD on low a_W foods. Generally, HPCD treatments are less effective on low a_W foods when compared to effects with liquid or high a_W foods (Garcia-Gonzalez et al., 2007). Individual studies reported discrepancies in treatment effects of supercritical HPCD against *E. coli* (Chen et al., 2017; Jung et al., 2009). This suggests that some of the treatment parameters

63 that are critical for the bactericidal effects have not been identified or controlled in the process
64 design.

65 Beef jerky is a ready-to-eat low aw product that caused outbreaks of foodborne disease (CDC,
66 1985; Eidson et al., 2000; Keene et al., 1997). The U.S Food Safety and Inspection Service requires
67 a 5-log reduction of *Salmonella* during production of beef jerky (USDA-FSIS, 2017). *E. coli* also
68 exhibits remarkable heat and desiccation resistance and FSIS requires a 5-log reduction of *E, coli*
69 in production of dry fermented sausages (Holck et al., 2011). However, following the FSIS
70 compliance guidelines for beef jerky production not only eliminates *Salmonella* but also decreases
71 product quality (Borowski et al., 2009; USDA-FSIS, 2017). Alternative processes to warrant the
72 safety of beef jerky can be validated to ensure a 5-log reduction of *Salmonella* and *E. coli*. Process
73 validation in food processing facilities is dependent on the availability of non-pathogenic surrogate
74 organisms for in-plant validation of process efficacy. Surrogate organisms must be validated with
75 relevant pathogens in the food matrix in which it will be used (Borowski et al., 2009; Garcia-
76 Hernandez et al., 2015; Kopit et al., 2014).

77 It was hypothesized that HPCD treatment reduces pathogens on beef jerky by 5 log and that *E. coli*
78 AW1.7, due to its remarkable resistance toward desiccation and heat (Mercer et al., 2015), is an
79 acceptable surrogate organism to represent STEC and *Salmonella*. Therefore, the objectives of this
80 research were to: (i) Identify conditions that can achieve a 5-log reduction of desiccated *E. coli*
81 and *S. enterica* with HPCD, (ii) Identify a non-pathogenic surrogate organism that displays equal
82 or higher resistance than these organisms and (iii) Validate the HPCD technology and the non-
83 pathogenic surrogate organism in beef jerky with a defined water activity.

2. Materials and Methods

2.1 Bacterial strains and culture conditions

Strains used in this study are listed in Table 1. All strains were maintained as 30% glycerol stock at -80 °C, and were incubated in liquid or on agar media at 37 °C for 16-18 h. Unless otherwise specified, *E. coli* and *Salmonella* were streaked onto Luria-Bertani (LB) (Fisher Scientific, Ottawa, ON) agar and incubated aerobically. A colony was inoculated into 5 mL LB broth and incubated aerobically with agitation (200 rpm). For heat treatments, *E. coli* AW1.7 and AW1.7 ΔpHR1, and *S.* Typhimurium ATCC 13311 pLHR and pRK 767 were grown in Tryptic Soy (TS) agar or broth (Difco™, Becton Dickinson, Mississauga, ON). *E. coli* AW1.7 and *S.* Typhimurium ATCC 13311 pLHR encode the locus of heat resistance (LHR), a genomic island that mediates resistance to wet heat, whereas *E. coli* AW1.7 ΔpHR1 and *S.* Typhimurium ATCC 13311 pRK767 do not. Growth media for *S.* Typhimurium ATCC 13311 pLHR and *S.* Typhimurium ATCC 13311 pRK767 were supplemented with 15 mg/L tetracycline-HCl to maintain the plasmid containing heat and tetracycline resistance genes (Seeras, 2017). Violet Red Bile Glucose Agar (VRBGA; Oxoid, Nepean, ON) incubated aerobically at 37 °C was used to differentiate between healthy and sublethally-injured cells. *S. carnosus* R6 FUA 2133 and *E. faecium* NRRL B-2354 were prepared similarly, but with TS agar or broth. For differentiation between healthy and sublethally-injured cells and for selective enrichment of *E. faecium*, mEnterococcus Agar (Difco) and aerobic incubation was used. *P. acidilactici* FUA 3072 was prepared similarly, but with De Man, Rogosa and Sharpe (MRS) agar or broth (Difco) and anaerobic incubation. All liquid subcultures were plated onto non-selective agar and incubated at 37 °C for 24 h. The resulting bacterial lawns were removed with 1 mL of sterile 0.1% peptone water (Difco). Cells were centrifuged at 9,000 x *g* for 2 min and resuspended in 1 mL of 0.1% peptone water to obtain the inoculum. To create a cocktail

of *E. coli*, the inoculum of 4 strains of STEC(03-2832 O121:H19, 05-6544 O26:H11, C0283 O157:H7, 03-6430 O145:NM) and 1 strain of enteropathogenic *E. coli* (PARC 449 O145:NM), were combined in equal volumes for a final volume of 3.5 mL and hereafter referred to as the STEC cocktail. All other strains were treated individually. All subsequent resuspension of samples and dilutions were done in 0.1% peptone water and all subsequent incubations were done at 37 °C for 16-18 h.

2.2 Preparation of beef jerky

Beef jerky was prepared using 2 beef inside rounds obtained from a federally inspected beef processing facility and were stored at -20 °C until use. Inside rounds (8.30 kg) were thawed at -1 °C until slightly frozen, sliced into 6 mm thick slices using a Berkel Model X13 meat slicer (Berkel, Chicago IL). Beef jerky seasoning (Unipac, Edmonton, AB) and cure COOAE1 (Newly Weds Foods, Edmonton, AB) were added according to manufacturer recommendations for the preparation of a marinated product, and sliced beef was marinated in the brine for 16-18 h at 4 °C. Slices were placed on racks, transferred into an ALKAR processing oven and smokehouse (ALKAR-RapidPak Inc., Lodi, WI, U.S.A.). Equipment settings for processing were as follows (dry bulb temperature / wet bulb temperature / relative humidity): 30 min at 35°C / 25°C / 45%; 60 min at 35°C / 24°C / 40%; 35°C / 25°C / 45%; 60 min at 45°C / 29°C / 30%; 60 min at 55°C / 35°C / 25%; 120 min at 80°C / 48°C / 20%. Auto Temperature probes monitored 4 areas in the smokehouse, and the a_W of beef jerky was measured with a water activity meter (CX-2, Aqualab by Meter, Pullman, WA) every 0.5 h after the first hour until the product reached a final a_W around 0.75. The beef jerky remained in the smokehouse for a total of 3.75 h with a final a_W of 0.74-0.75. After processing, beef jerky was double bagged in 3 mm and 4 mm vacuum bags (Unipac, Edmonton, AB) with 5 slices per bag and sealed using a vacuum packager (Multivac Inc. Model

C200, Wolfertschwenden, Germany). Samples were stored at 0 °C until further use. Microbial contamination of the uninoculated beef jerky was determined by plating onto LB agar; no contamination was detected.

2.3 Sample preparation

2.3.1 Preparation of desiccated bacteria for HPCD and heat treatments.

To prepare samples for heat treatments, samples (20 µL) of *E. coli* AW1.7, *E. coli* AW1.7 ΔpHR1, *S.* Typhimurium ATCC 13311 pLHR, *S.* Typhimurium ATCC 13311 pRK 767, *Salmonella* FUA 1934, *Salmonella* FUA 1946 and *Salmonella* FUA 1955 were dried in glass vials (12x30 mm; Supelco, Sigma Aldrich, Oakville, ON). For HPCD treatments, samples (20 µL) were dried in individual lids removed from 1.5 mL Eppendorf tubes (Fisher Scientific) placed in a biosafety cabinet for 6-8 h. Initial cell counts of the liquid inoculum ranged from 10-11 log CFU/mL. After drying, samples were stored for 16-18 h at 37 °C in air-tight containers with different desiccants to equilibrate samples to a specific a_w (Fontana, 2008). Cell counts from one set of samples were determined after drying. Dried samples for heating were exposed to 60 °C for 1, 4 or 15 min using a water bath (Thermo Scientific NESLAB EX 7, Waltham, MA). Remaining samples were treated with HPCD using the Mobile CO_2 Pasteurization Apparatus as described in Section 2.4. The cell counts were determined after each treatment.

2.3.2 Preparation of beef jerky samples for HPCD treatments

Whole beef jerky slices were cut into rectangular samples with an average surface area of 2 cm^2. Each sample was vortexed in the inoculum for 30 s to inoculate the surface. After inoculation, samples were air-dried for 10 min in a biosafety cabinet to remove surface moisture. Initial cell counts of each strain after inoculation were determined by resuspending one sample in 1 mL of 0.1% peptone water, vortexing twice for 20 s with a 1-min rest period between each vortex,

followed by dilution and plating. Initial cell counts on beef jerky ranged from 9-10 log CFU/2 cm^2. The remaining beef jerky samples were transferred to an air-tight container with either a saturated solution of sodium chloride or water and stored at 22 °C for 2 weeks or 1 week to equilibrate the samples to a_W 0.75 or 0.9, respectively. Cell counts from one set of samples were determined after equilibration to determine the reduction of cell counts after the equilibration and drying process. Remaining samples were treated with HPCD or pressurized N_2 in the Mobile CO_2 Pasteurization Apparatus as described in Section 2.4. Cell counts were determined after each treatment. For samples inoculated with *E. coli* and *Salmonella*, total viable counts were determined by surface plating on TS agar; cell counts were additionally determined on VRBGA, which excludes sublethally injured cells. The same comparison was done for samples inoculated with *E. faecium* but with TS agar and mEnterococcus agar. Beef jerky samples inoculated with *E. coli*, *Salmonella*, and *E. faecium* were also analysed after non-selective enrichment to detect growth when post-treatment cell counts were below the detection limit (1 log CFU/2 cm^2). Samples were incubated in TS broth at 37 °C for 16-18 h. The overnight enrichments were streaked onto TS agar and VRBGA or mEnterococcus agar for samples inoculated with *E. coli* and *Salmonella*, or *E. faecium*, respectively.

2.4 Mobile CO_2 Pasteurization Apparatus

Figure 1 shows the schematic diagram of the custom-built Mobile CO_2 Pasteurization Apparatus. Briefly, a CO_2 or N_2 cylinder with a syphon for liquid withdrawal was connected to a syringe pump (Model 260D, Teledyne ISCO Inc., Lincoln, NE). The syringe pump was cooled with a refrigeration bath (Lauda Alpha R8, Delran, NJ) to ensure the CO_2 remained in the liquid phase for compression. CO_2 or N_2 flowed into the vessel (V_i = 42.53 mL) and electrical heating bands (Brisk Heat, Columbus, OH) surrounded the vessel, as well as the lines before and after the vessel

to heat the system to the desired temperature. Temperature controllers from Maxthermo (MC-2438, Taipei, Taiwan) or Omega (CN7500, Spectris Canada, St-Eustache, QC) connected to thermocouples installed before and after the vessel or in the vessel, respectively, were used to control the electrical heating bands. Pressure gauges were from Swagelok (Cleveland, OH, USA). A micro-metering valve (SLPMMV46V, Butech, Erie, PA, USA) was installed at the vessel outlet to manually control the depressurization rate. An in-line 0.2 μm pore filter was installed after the micro-metering valve to prevent bacterial contamination. A metal wire basket with 3 shelves was constructed for simultaneous treatment of multiple samples during one cycle and was dipped in 100% ethanol and flamed between treatments. Sterile water was pipetted onto filter paper (Whatman, Pore Size: 8 μm, 110 mm diameter, Sigma Aldrich, Oakville, ON, Canada), which was placed on the top shelf of the sample basket, directly below the CO_2 inlet. Sufficient water was added to ensure that the gaseous environment in the vessel was saturated with water. Considering the solubility of water in CO_2 and N_2 at 65 °C and 5.7 MPa, 30 μL water were added to treatments with CO_2 and 105 μL water were added to treatments with N_2 (Hou et al., 2013; Rigby and Prausnitz, 1968). Samples were placed on the middle or bottom shelves of the basket with one sample per shelf. The basket was placed into the vessel, which was preheated to 65 °C. The system was pressurized to 5.7 MPa or 12.0 MPa and treatments were conducted at 65 °C for 1, 4, 8, or 15 min. The treatment time was considered to start after target pressure was reached and all valves were closed. After treatments, the system was depressurized at a rate of 6 MPa/min. The samples were then removed from the vessel for microbiological analysis.

2.5 Statistical analysis

All experiments were conducted in triplicate as independent experiments. Raw data were log transformed and the reduction of cell counts was calculated by subtracting log cell counts after

199 treatments from initial log cell counts. Data were subjected to analysis of variance (ANOVA) using
200 the PROC GLM procedure of the University Edition of SAS software (SAS Institute Inc., Cary,
201 NC). Tukey's posthoc test was used to determine differences among means. Significant differences
202 were assessed at an error probability of 5% (P<0.05).

203 **3. Results**

204 **3.1 Effect of heat treatment on desiccated cells**

205 To allow attribution of process lethalities to CO_2 treatments, initial experiments quantified the
206 combined lethal effect of drying, equilibration to different water activities, and heating at 60 °C
207 on strains of *E. coli* and *Salmonella*. At a_W of less than 0.9, the combined lethality of drying,
208 equilibration, and heating to 60 °C was less than 2 log(cfu/g) in case of the waste water isolates of
209 *Salmonella*, and less than 3 log(cfu/g) for all other strains (Figs. S1 and S2). At a_W between 0.9
210 and 0.99, the combined lethality of drying, equilibration, and heating to 60 °C ranged from less
211 than 2 log(cfu/g) to more than 8 log(cfu/g). *S.* Typhimurium ATCC 13311 pLHR, which is among
212 the strains with the highest resistance to wet heat, was the most sensitive to heating after
213 desiccation (Fig. S1).

214 **3.2 Influence of water addition and process time on the lethality of HPCD**

215 Due to the significance of water in the inactivation mechanism of HPCD, cells of *E. coli* were
216 treated with dry CO_2 or with water-saturated CO_2. The lethality of drying or treatment with dry
217 CO_2 at 5.7 MPa and 65 °C for 15 min was less than 2 log(cfu/g) for wild type strains of *E. coli*,
218 and less than 5 log(cfu/g) for the laboratory strain *E. coli* MG1655 (Fig. 2). Treatment with water-
219 saturated CO_2 reduced cell counts of all strains by 6 to more than 8 log(cfu/g). These results
220 highlight the critical role of water for the lethality of pressurized CO_2.

To determine the impact of treatment time on process lethality, *E. coli* AW1.7 and MG1655 and the STEC cocktail were treated with water-saturated CO_2 at 5.7 MPa and 65 °C for 4, 8 or 15 min (Fig. 3). The treatment time did not influence the process lethality (Fig. 3). The effect of treatment time was also analysed with an extended set of strains including 5 strains of *Salmonella* as well as *P. acidilactici*, *E. faecium* and *S. carnosus*, which were included to assess their suitability as surrogate organisms for pathogens. Treatment time significantly influenced the process lethality for *S. enterica* FUA1934 and FUA1955 (Table 2); for other strains, the effect of time was not significant. The waste-water isolates of *Salmonella* were most resistant to HPCD treatment (Table 2). *Pediococcus* was previously used as a surrogate organism for *E. coli* and *Salmonella* in beef jerky production (Borowski et al., 2009); however, *P. acidilactici* FUA3072 was more sensitive to HPCD than any of the waste-water isolates of *Salmonella* (Table 2). *E. faecium* NRRL B-2354 has been used as a surrogate for *Salmonella* in other dry foods (Kopit et al., 2014). The resistance of *E. faecium* NRRL B-2354 to HPCD was higher when compared to the resistance of *S.* Typhimurium ATCC 13311 and *S.* Senftenberg ATCC 43845 and approximately equivalent to the waste-water isolates of *Salmonella* (Table 2). Because *E. faecium* is assessed as a biosafety level 2 organism in some jurisdictions, *S. carnosus* FUA2133, a food starter culture organism (Tang et al., 2018), was additionally analysed. The resistance of *S. carnosus* was equal or higher when compared to any other organism and the process lethality against *S. carnosus* was 2 log(cfu/g) or less for all treatments (Table 2).

3.3 Survival of desiccated bacteria inoculated onto beef jerky and treated with HPCD

To validate the antimicrobial effect of HPCD in food, the target pathogens and surrogate organisms were inoculated onto beef jerky, equilibrated to a_W 0.75, and treated with water-saturated CO_2 at

5.7 MPa and 65 °C for 15 min. Counts of the *E. coli* strains tested were below the detection limit after treatment and non-selective enrichment did not recover sublethally injured cells (Fig. 4).

Treatment of *Salmonella* inoculated onto beef jerky demonstrated that strains of *Salmonella* displayed a higher resistance and a larger variability in cell counts after treatment with saturated CO_2 at 5.7 MPa and 65 °C (Fig. 5A). Treatment time did not impact treatment lethality when results were compared to that of the treatment of dry cells; however, treatment lethality of cells on beef jerky was higher when compared to the treatments lethality on dry cells (Table 2 and Fig. 5A). Treatments on beef jerky eliminated only 2 of the 5 strains of *Salmonella*; for the remaining 3 strains, cell counts after 15 min of treatment were above the detection limit, or viable cells were recovered by non-selective enrichment (Fig. 5A). *E. faecium* and *S. carnosus* were more resistant than *Salmonella* (Fig. 5A). To determine whether the treatment lethality is enhanced by a higher water activity, treatments were also carried out with beef jerky samples that were equilibrated to a_W 0.9 (Fig. 5B). Increasing the water activity of beef jerky did not substantially enhance treatment lethality (Figs. 5A and 5B). Similarly, treatment with a pressure of 12.0 MPa CO_2, corresponding to treatment in the supercritical region of the phase diagram, did not increase treatment lethality (Figs. 5A and 5C).

3.4 Survival of desiccated bacteria inoculated onto beef jerky and treated with pressurized N_2

To determine whether the lethality of treatments with HPCD relates to specific properties of water-saturated CO_2 or is mainly attributable to the combination of pressure and temperature, experiments were also conducted with pressurized N_2 as an inert gas. Experiments were carried out with strains exhibiting low, intermediate and high resistance to HPCD, i.e. *S.* Typhimurium ATCC 13311, *Salmonella* FUA1934 and *S. carnosus* (Fig. 6), and with beef jerky that was adjusted

to a_w of 0.75 (Fig. 6A) or 0.9 (Fig. 6B). Cell counts of *S. carnosus* were unaffected by any of the treatments; cell counts of *Salmonella* FUA1934 were reduced by less than 3 log(cfu/cm^2) while cell counts of *S.* Typhimurium were reduced by about 8 log(cfu/cm^2). These results demonstrate that CO_2 substantially contributes to the treatment lethality (compare Figs. 5A and 5B with Figs. 6A and 6B).

4. Discussion

4.1 Effect of water and treatment time on the reduction of *E. coli* treated with HPCD

HPCD is not effective to reduce cell counts of dry bacteria (Chen et al., 2017; Garcia-Gonzalez et al., 2007) because CO_2 cannot dissociate to carbonic acid without water, thus preventing extra- and intracellular acidification. Additionally, cell walls are more permeable to CO_2 with moisture, resulting in increased absorption (Kumagai et al., 1997) and diffusion (Dillow et al., 1999). The unprecedented use of dry versus water-saturated CO_2 in the current study demonstrated that CO_2 is bactericidal only when combined with water. Treatment of dry cells at a_W 0.75 with water-saturated CO_2 resulted in a > 7 log reduction.

In the current study, treatment time did not substantially affect the reduction of desiccated bacterial cells. Some but not all of the past studies reported an influence of treatment time on the lethality of HPCD (Bae et al., 2011; Debs-Louka et al., 1999). When the initial cell counts were higher, a longer HPCD treatment time was needed to achieve the same reduction as when starting with lower counts (Erkmen, 2000). In this study, initial bacterial cell counts were high, which may contribute toward the resistance of cells. However, Uesugi et al. (2006) observed similar reductions of *Salmonella* on almonds regardless of the initial cell counts.

4.2 Effect of the food matrix on the reduction of *E. coli* and *Salmonella*

Bacteria were more sensitive to HPCD treatment when inoculated onto beef jerky compared to treatment of desiccated cells. These results are counter-intuitive as many past studies suggest that food matrices exert protective effects on bacterial cells (Garcia-Gonzalez et al., 2007; Sirisee et al., 1998). This protective effect may relate to the presence of fat (Garcia-Gonzalez et al., 2009; Metrick et al., 1989) or proteins (Debs-Louka et al., 1999; Metrick et al., 1989). However, past reports on the protective effects of the food matrix all relate to high-a_W foods. It is likely that the effect of the food matrix that was observed in the current study predominantly relates to the moisture content and the sorption isotherm of beef jerky. Even though the desiccated cells and beef jerky were equilibrated to the same a_w, beef jerky probably retained more moisture during treatments compared to dried cells, resulting in increased sensitivity of bacteria on beef jerky. In a parallel study, a shift in the water sorption isotherms of beef jerky under HPCD conditions was observed (Ren, 2019), where the moisture content increased in a steep manner at a_w of 0.73 at 5.7 MPa and 45 ^oC. Therefore, bacterial cells on beef jerky samples were exposed to higher water activity when compared to treatments of dried bacterial cultures.

4.3. Effect of CO_2 phase on the reduction of *Salmonella* and *S. carnosus* treated with HPCD

For treatments of high a_W foods, the lethality of supercritical CO_2 is higher when compared to treatments with gas-phase CO_2 (Hong and Pyun, 1999; Isenschmid et al., 1995); an increase of treatment pressure typically resulted in an increased lethality (Bae et al., 2011; Damar and Balaban, 2006). Mapping the treatment lethality of HPCD at different combinations of pressure and temperature demonstrated that dry cells were more effectively eliminated by treatment with gas-phase CO_2 in combination with elevated temperature (Chen et al., 2017). Gas-phase CO_2 has increased mass transport properties and a lower density, and thus diffuses into the cell faster (Chen et al., 2017). Higher temperature further increases the diffusivity of CO_2 and the fluidity of the cell

311 membrane (Damar and Balaban, 2006). The current study confirmed for a broad range of Gram-
312 negative and Gram-positive target organisms that gaseous CO_2 was more effective than
313 supercritical CO_2 to inactivate bacteria inoculated onto low a_W foods.

314 The specific contribution of CO_2 to bacterial inactivation was probed by assessment of the effect
315 of treatments at elevated temperature and ambient pressure, and by treatments at elevated
316 temperature and high N_2 pressure. Treatments at elevated temperature and ambient pressure had
317 only minimal bactericidal effect if the water activity was below 0.9, confirming the exceptional
318 heat resistance of dry *E. coli* and *Salmonella* (Finn et al., 2013; Li and Gänzle, 2016). The current
319 study demonstrated that the lethality of treatments with supercritical N_2 was higher when compared
320 to treatments at ambient pressure, but lower when compared to treatments with gas-phase CO_2 at
321 the same pressure and temperature. Supercritical N_2 was also less effective than supercritical CO_2
322 in reducing cell counts of yeast, *E. coli* or *Salmonella* at a high water activity (Dillow et al., 1999;
323 Nakamura et al., 1994), likely due to the low solubility of N_2 in water (Vo et al., 2013). Unlike
324 CO_2, N_2 interaction with water does not result in the release of protons that reduce the pH.
325 Therefore, the lethality of high pressure N_2 treatments is strictly related to physical factors, such
326 as pressure-induced damage to the cell membrane. CO_2 treatments are more effective due to the
327 combined effects of pressure, solubilization and acidification.

328 **4.4 Resistance of *E. coli, Salmonella,* and potential surrogate organisms to HPCD**

329 The current study compared the sensitivity of *E. coli* and *Salmonella* to several potential surrogate
330 organisms. HPCD treatment results in sublethal injury of surviving cells (Bi et al., 2015; Garcia-
331 Gonzalez et al., 2007; Sirisee et al., 1998), which may cause an over-estimation of treatment
332 efficacy (Zhao et al., 2013). Sublethal injury of surviving cells was also indicated by comparison
333 of cell counts on selective and non-selective media, and by a variable colony size after HPCD

334 treatments (this study; Isenschmid et al., 1995). To ensure an accurate assessment of treatment
335 lethality, non-selective enrichments were conducted for beef jerky samples to allow for recovery
336 of sublethally injured cells prior to detection on selective media.

337 A suitable surrogate organism exhibits resistance that is greater than or equal to that of the target
338 organism, and must be evaluated in the food system in which it will be used (Borowski et al.,
339 2009). The resistance of *E. coli* AW1.7 was greater or equal to the resistance of the STEC cocktail
340 at all tested conditions; *E. coli* AW1.7 is thus a suitable surrogate organism for STEC after
341 treatment of beef jerky with HPCD. The resistance of strains of *Salmonella* to drying and treatment
342 with HPCD, however, was substantially higher than the resistance of strains of *E. coli*; this
343 confirms and extends prior reports on the resistance of *E. coli* and *Salmonella* to drying and dry
344 storage (Seeras, 2017). The selection of strains of *Salmonella* was initially guided by the
345 hypothesis that the locus of heat resistance, which confers resistance to wet heat to *Salmonella* and
346 *E. coli* (Mercer et al., 2017, 2015), also confers resistance to HPCD treatments at elevated
347 temperature. The resistance of the LHR-positive *S.* Senftenberg ATCC43845 to drying and dry
348 heat, however, was approximately equivalent to the resistance of the LHR-negative *S.*
349 Typhimurium ATCC 13311. Together with prior data on the resistance of LHR-positive and LHR-
350 negative *E. coli* to drying, dry heat, and HPCD (Chen et al., 2017), these results document that the
351 LHR does not increase heat resistance of dry cells, or the resistance to treatment with HPCD.

352 Waste-water isolates of *Salmonella enterica* exhibited the highest resistance to drying and HPCD.
353 The selection of strains was motivated by the observation that waste-water isolates of *E. coli* are
354 highly resistant to chlorine and other physical and chemical stressors (Wang et al., 2020; Zhi et
355 al., 2016). At this time, little is known about the genetic or physiological properties of the waste-
356 water isolates of *Salmonella*. Preliminary experiments indicate that these strains are also resistant

to dry heat (Gautam, 2019). Their isolation from municipal waste water indicates that these strains originate from human-related environments, and may also contaminate food.

Pediococcus spp. was used as a surrogate organism in beef jerky to predict the reduction of *E. coli* O157:H7 and *Salmonella* (Borowski et al., 2009). The resistance of *P. acidilactici* FUA3072 to HPCD matched the resistance of the STEC cocktail and *S.* Typhimurium ATCC 13311 but the strain was more sensitive than several other strains of *Salmonella*, making it unsuitable as a surrogate organism. *E. faecium* NRRL B-2354 was validated as a surrogate to represent *Salmonella* on almonds, and its heat resistance was comparable to *S.* Enteritidis (Kopit et al., 2014). *E. faecium* was consistently more resistant to HPCD than *Salmonella* (this study), making it an suitable choice as a surrogate organism. *E. faecium* NRRL B-2354 is listed as biosafety level 1 organism by the American Type Culture Collection (Kopit et al., 2014); however, other jurisdictions classify all strains of *E. faecium* as biosafety level 2 organisms, which prevents the use of *E. faecium* NRRL B-2354 as surrogate organism in food processing plants. Therefore, *S. carnosus* FUA 2133, an isolate from a meat starter culture, was investigated as an alternative surrogate strain. The resistance of *S. carnosus* FUA 2133 to HPCD treatments was comparable to *E. faecium* and it was consistently more resistant than *Salmonella*. Therefore, *S. carnosus* FUA 2133 is a suitable surrogate organism because it showed greater resistance to the treatments than the target organisms.

In conclusion, the current study achieved inactivation of desiccated STEC and *Salmonella* by more than 5 log (cfu/cm^2) by using HPCD treatments. In addition, *E. faecium* NRRL B-2354 and *S. carnosus* were validated as surrogate organisms for *Salmonella*. In contrast to high a_W food, low a_W foods are more effectively treated with water-saturated gaseous CO_2. A comparison of the bactericidal effect of water-saturated high pressure CO_2 (this study) with the effect of the same

treatment on color and texture of beef jerky (Ren, 2019) demonstrated that treatment parameters for inactivation of STEC and *Salmonella* have a minimal impact on product quality. Moreover, the treatment of beef jerky could incorporate HPCD treatments into the drying process to facilitate high inactivation while the a_W remains high, followed by a drying step to achieve the desired a_W of the product.

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References

Aslam, M., Greer, G.G., Nattress, F.M., Gill, C.O., McMullen, L.M., 2004. Genotypic analysis of *Escherichia coli* recovered from product and equipment at a beef-packing plant. J. Appl. Microbiol. 97, 78–86.

Bae, Y.Y., Kim, N.H., Kim, K.H., Kim, B.C., Rhee, M.S., 2011. Supercritical carbon dioxide as a potential intervention for ground pork decontamination. J. Food Saf. 31, 48–53.

Baylis, C.L., MacPhee, S., Robinson, A.J., Griffiths, R., Lilley, K., Betts, R.P., 2004. Survival of *Escherichia coli* O157:H7, O111:H− and O26:H11 in artificially contaminated chocolate and confectionery products. Int. J. Food Microbiol. 96, 35–48. https://doi.org/10.1016/j.ijfoodmicro.2004.03.007

Bi, X., Wang, Y., Zhao, F., Sun, Z., Hu, X., Liao, X., 2015. Sublethal injury and recovery of *Escherichia coli* O157: H7 by high pressure carbon dioxide. Food Control 50, 705–713. https://doi.org/10.1016/j.foodcont.2014.10.014

Borowski, A.G., Ingham, S.C., Ingham, B.H., 2009. Validation of ground-and-formed beef jerky

processes using commercial lactic acid bacteria starter cultures as pathogen surrogates. J. Food Prot. 72, 1234–1247. https://doi.org/10.4315/0362-028X-72.6.1234

Calhoun, C.L., Frazier, W.C., 1966. Effect of available water on thermal resistance of three nonsporeforming species of bacteria. Appl. Microbiol. 14, 416–420.

Chen, Y.Y., Temelli, F., Gänzle, M.G., 2017. Mechanisms of inactivation of dry *Escherichia coli* by high-pressure carbon dioxide. Appl. Environ. Microbiol. 83, e00062-17. https://doi.org/10.1128/aem.00062-17

Damar, S., Balaban, M.O., 2006. Review of dense phase CO_2 technology: Microbial and enzyme inactivation, and effects on food quality. J. Food Sci. 71, R1–R11. https://doi.org/10.1111/j.1365-2621.2006.tb12397.x

Debs-Louka, E., Louka, N., Abraham, G., Chabot, V., Allaf, K., 1999. Effect of compressed carbon dioxide on microbial cell viability. Appl. Environ. Microbiol. 65, 626–631.

Dillow, A.K., Dehghani, F., Hrkach, J.S., Foster, N.R., Langer, R., 1999. Bacterial inactivation by using near- and supercritical carbon dioxide. Proc. Natl. Acad. Sci. 96, 10344–10348. https://doi.org/10.1073/pnas.96.18.10344

Erkmen, O., 2000. Inactivation of *Salmonella typhimurium* by high pressure carbon dioxide. Food Microbiol. 17, 225–232. https://doi.org/10.1006/fmic.1999.0308

Finn, S., Condell, O., McClure, P., Amézquita, A., Fanning, S., 2013. Mechanisms of survival, responses, and sources of *Salmonella* in low-moisture environments. Front. Microbiol. 4, 331. https://doi.org/10.3389/fmicb.2013.00331

Fontana, A.J., 2008. Appendix A: Water activity of saturated salt solutions, in: Water Activity in Foods. pp. 391–393. https://doi.org/10.1002/9780470376454.app1

Garcia-Gonzalez, L., Geeraerd, A.H., Elst, K., Van Ginneken, L., Van Impe, J.F., Devlieghere,

426 F., 2009. Influence of type of microorganism, food ingredients and food properties on high-
427 pressure carbon dioxide inactivation of microorganisms. Int. J. Food Microbiol. 129, 253–
428 263. https://doi.org/10.1016/j.ijfoodmicro.2008.12.005
429 Garcia-Gonzalez, L., Geeraerd, A.H., Spilimbergo, S., Elst, K., Van Ginneken, L., Debevere, J.,
430 Van Impe, J.F., Devlieghere, F., 2007. High pressure carbon dioxide inactivation of
431 microorganisms in foods: The past, the present and the future. Int. J. Food Microbiol. 117,
432 1–28. https://doi.org/10.1016/j.ijfoodmicro.2007.02.018
433 Garcia-Hernandez, R., McMullen, L., Gänzle, M.G., 2015. Development and validation of a
434 surrogate strain cocktail to evaluate bactericidal effects of pressure on verotoxigenic
435 *Escherichia coli*. Int. J. Food Microbiol. 205, 16–22.
436 https://doi.org/10.1016/j.ijfoodmicro.2015.03.028
437 Gautam, B., 2019. Influence of water activity on thermal resistance of *Salmonella enterica* and
438 quality changes in low-moisture foods. https://doi.org/10.7939/R3-A8GY-MS37
439 Goepfert, J.M., Iskander, I.K., Amundson, C.H., 1970. Relation of the heat resistance of
440 salmonellae to the water activity of the environment. Appl. Microbiol. 19, 429–433.
441 https://doi.org/10.1016/S0378-3812(01)00464-2
442 Gruzdev, N., Pinto, R., Sela Saldinger, S., 2012. Persistence of *Salmonella enterica* during
443 dehydration and subsequent cold storage. Food Microbiol. 32, 415–422.
444 https://doi.org/10.1016/j.fm.2012.08.003
445 Hauben, K.J.A., Bartlett, D.H., Soontjens, C.C.F., Cornelis, K., Wuytack, E.Y., Michiels, C.W.,
446 1997. *Escherichia coli* mutants resistant to inactivation by high hydrostatic pressure. Appl.
447 Environ. Microbiol. 63, 945–950. https://doi.org/10.1016/j.meegid.2012.03.025
448 Health Canada, 2016. Yearly foodborne illness estimates for Canada [WWW Document]. URL

https://www.canada.ca/en/public-health/services/food-borne-illness-canada/yearly-food-borne-illness-estimates-canada.html (accessed 2.25.19).

Holck, A.L., Axelsson, L., Rode, T.M., Høy, M., Måge, I., Alvseike, O., L'Abée-Lund, T.M., Omer, M.K., Granum, P.E., Heir, E., 2011. Reduction of verotoxigenic *Escherichia coli* in production of fermented sausages. Meat Sci. 89, 286–295. https://doi.org/10.1016/J.MEATSCI.2011.04.031

Hong, S.I., Pyun, Y.R., 1999. Inactivation kinetics of *Lactobacillus plantarum* by high pressure carbon dioxide. J. Food Sci. 64, 728–733. https://doi.org/10.1111/j.1365-2621.1999.tb15120.x

Hou, S., Maitland, G.C., Trusler, J.P.M., 2013. Measurement and modeling of the phase behavior of the (carbon dioxide + water) mixture at temperatures from 298.15 K to 448.15 K. J. Supercrit. Fluids 73, 87–96. https://doi.org/10.1016/j.supflu.2012.11.011

Isenschmid, A., Marison, I.W., von Stockar, U., 1995. The influence of pressure and temperature of compressed CO_2 on the survival of yeast cells. J. Biotechnol. 39, 229–237. https://doi.org/10.1016/0168-1656(95)00018-L

Jung, W.Y., Choi, Y.M., Rhee, M.S., 2009. Potential use of supercritical carbon dioxide to decontaminate *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella typhimurium* in alfalfa sprouted seeds. Int. J. Food Microbiol. 136, 66–70. https://doi.org/10.1016/j.ijfoodmicro.2009.08.014

Kopit, L.M., Kim, E.B., Siezen, R.J., Harris, L.J., Marco, M.L., 2014. Safety of the surrogate microorganism *Enterococcus faecium* NRRL B-2354 for use in thermal process validation. Appl. Environ. Microbiol. 80, 1899–1909. https://doi.org/10.1128/AEM.03859-13

Kumagai, H., Hata, C., Nakamura, K., 1997. CO_2 sorption by microbial cells and sterilization by

high-pressure CO_2. Biosci. Biotechnol. Biochem. 61, 931–935. https://doi.org/10.1271/bbb.61.931

Li, H., Gänzle, M., 2016. Some like it hot: Heat resistance of *Escherichia coli* in food. Front. Microbiol. 7, 01763. https://doi.org/10.3389/fmicb.2016.01763

Lin, H. -M, Yang, Z., Chen, L. -F, 1992. Inactivation of *Saccharomyces cerevisiae* by supercritical and subcritical carbon dioxide. Biotechnol. Prog. 8, 458–461. https://doi.org/10.1021/bp00017a013

Liu, Y., Gill, A., McMullen, L., Gänzle, M.G., 2015. Variation in heat and pressure resistance of verotoxigenic and nontoxigenic *Escherichia coli*. J. Food Prot. 78, 111–120. https://doi.org/10.4315/0362-028X.JFP-14-267

Mercer, R.G., Walker, B.D., Yang, X., McMullen, L.M., Gänzle, M.G., 2017. The locus of heat resistance (LHR) mediates heat resistance in *Salmonella enterica*, *Escherichia coli* and *Enterobacter cloacae*. Food Microbiol. 64, 96–103. https://doi.org/10.1016/j.fm.2016.12.018

Mercer, R.G., Zheng, J., Garcia-Hernandez, R., Ruan, L., Gänzle, M.G., McMullen, L.M., 2015. Genetic determinants of heat resistance in *Escherichia coli*. Front. Microbiol. 6, 932. https://doi.org/10.3389/fmicb.2015.00932

Metrick, C., Hoover, D.G., Farkas, D.F., 1989. Effects of high hydrostatic pressure on heat-resistant and heat-sensitive strains of *Salmonella*. J. Food Sci. 54, 1547–1549. https://doi.org/10.1111/j.1365-2621.1989.tb05156.x

Nakamura, K., Enomoto, A., Fukushima, H., Nagai, K., Hakoda, M., 1994. Disruption of microbial cells by the flash discharge of high-pressure carbon dioxide. Biosci. Biotechnol. Biochem. 58, 1297–1301. https://doi.org/10.1080/bbb.58.1297

Pan, Z., Bingol, G., Brandl, M.T., McHugh, T.H., 2012. Review of current technologies for reduction of *Salmonella* populations on almonds. Food Bioprocess Technol. 5, 2046–2057. https://doi.org/10.1007/s11947-012-0789-6

Pleitner, A., Zhai, Y., Winter, R., Ruan, L., McMullen, L.M.L.M., Gänzle, M.G.M.G., 2012. Compatible solutes contribute to heat resistance and ribosome stability in *Escherichia coli* AW1.7. Biochim. Biophys. Acta - Proteins Proteomics 1824, 1351–1357. https://doi.org/10.1016/j.bbapap.2012.07.007

Ren, Y., 2019. Effect of high pressure CO_2 treatment on the moisture sorption isotherm and physicochemical properties of beef jerky. University of Alberta. https://doi.org/10.7939/R3-PPG3-WR71

Rigby, M., Prausnitz, J.M., 1968. Solubility of water in compressed nitrogen, argon, and methane. J. Phys. Chem. 72, 330–334. https://doi.org/10.1021/j100847a064

Seeras, A., 2017. Survival and persistence of dried *Salmonella enterica* in low water activity conditions. University of Alberta. https://doi.org/https://doi.org/10.7939/R3TM72C98

Sirisee, U., Hsieh, F., Huff, H.E., 1998. Microbial safety of supercritical carbon dioxide processes. J. Food Process. Preserv. 22, 387–403. https://doi.org/10.1111/j.1745-4549.1998.tb00358.x

Tang, K.X., Shi, T., Gänzle, M.G., 2018. Effect of starter cultures on taste-active amino acids and survival of pathogenic *Escherichia coli* in dry fermented beef sausages. Eur. Food Res. Technol. 244, 2203–2212. https://doi.org/10.1007/s00217-018-3130-4

Uesugi, A.R., Danyluk, M.D., Harris, L.J., 2006. Survival of *Salmonella enteritidis* phage type 30 on inoculated almonds stored at -20, 4, 23, and 35°C. J. Food Prot. 69, 1851–1857. https://doi.org/10.4315/0362-028X-69.8.1851

518 USDA -FSIS, 2017. *Salmonella* compliance guidelines for small and very small meat and
519 poultry establishments that produce ready-to-eat (RTE) products and revised Appendix A
520 June 2017 [WWW Document]. URL
521 https://www.fsis.usda.gov/wps/wcm/connect/bf3f01a1-a0b7-4902-a2df-
522 a87c73d1b633/Salmonella-Compliance-Guideline-SVSP-RTE-Appendix-
523 A.pdf?MOD=AJPERES (accessed 9.29.19).
524 Vo, H.T., Imai, T., Teeka, J., Sekine, M., Kanno, A., Van Le, T., Higuchi, T., Phummala, K.,
525 Yamamoto, K., 2013. Comparison of disinfection effect of pressurized gases of CO_2, N_2O,
526 and N_2 on *Escherichia coli*. Water Res. 47, 4286–4293.
527 https://doi.org/10.1016/j.watres.2013.04.053
528 Wang, Z., Fang, Y., Zhi, S., Simpson, D.J., Gill, A., McMullen, L.M., Neumann, N.F., Gänzle,
529 M.G., 2020. The locus of heat resistance confers resistance to chlorine and other oxidizing
530 chemicals in *Escherichia coli*. Appl. Environ. Microbiol. in press.
531 https://doi.org/10.1128/AEM.02123-19
532 Wei, C.I., Balaban, M.O., Fernando, S.Y., Peplow, A.J., 1991. Bacterial effect of high pressure
533 CO_2 treatment on foods spiked with *Listeria* or *Salmonella*. J. Food Prot. 54, 189–193.
534 https://doi.org/10.4315/0362-028x-54.3.189
535 Winter, A.R., Stewart, G.F., McFarlane, V.H., Solowey, M., 1946. Pasteurization of liquid egg
536 products III. Destruction of *Salmonella* in liquid whole egg. Am. J. Public Health 36, 451–
537 460.
538 Zhao, F., Bi, X., Hao, Y., Liao, X., 2013. Induction of viable but nonculturable *Escherichia coli*
539 O157:H7 by high pressure CO_2 and its characteristics. PLoS One 8, e62388.
540 https://doi.org/10.1371/journal.pone.0062388

Zhi, S., Banting, G., Li, Q., Edge, T.A., Topp, E., Sokurenko, M., Scott, C., Braithwaite, S., Ruecker, N.J., Yasui, Y., McAllister, T., Chui, L., Neumann, N.F., 2016. Evidence of naturalized stress-tolerant strains of *Escherichia coli* in municipal wastewater treatment plants. Appl. Environ. Microbiol. 82, 5505–5518. https://doi.org/10.1128/AEM.00143-16

Figure legends

Figure 1. Schematic diagram of the Mobile CO_2 Pasteurization Apparatus (MPA). V1 – V4, needle valves; CV1 – CV2, check valves; PI, pressure gauges; RV, safety relief valve; MMV, micrometering valve; TC, thermocouple.

Figure 2. Effect of water on the reduction of *E. coli* AW1.7 (black bars), *E. coli* MG1655 (grey bars), and the STEC cocktail (white bars) desiccated to a_W 0.75, followed by CO_2 treatment at 5.7 MPa and 65 °C for 15 min. In the "Dry CO_2" treatment no water was added to the vessel. In the "Saturated CO_2", water was added to saturate gaseous CO_2. Data are shown as means ± standard deviation (n=3-6). Letters a-c denote significant differences among different treatments for the same strain ($P<0.05$).

Figure 3. Effect of treatment time on the lethality of desiccation, equilibration, and treatment with HPCD on *E. coli* AW1.7 (black bars), *E. coli* MG1655 (grey bars) and the STEC cocktail (white bars). Cells were desiccated and equilibrated to a_W 0.75, followed by treatment with water-saturated CO_2 at 5.7 MPa and 65 °C. Data are shown as means ± standard deviation (n=3).

Figure 4. Effect of water-saturated CO_2 at 5.7 MPa and 65 °C for 15 min on the reduction of *E. coli* AW1.7 (black bars), *E. coli* MG1655 (grey bars), and the STEC cocktail (white bars) inoculated onto beef jerky and equilibrated to a_W 0.75. Y-axis was limited to detection limit. * indicates no growth after enrichment. Data are shown as means ± standard deviation (n=3).

Figure 5. Reduction of *S.* Typhimurium ATCC 13311 (black bars), *S.* Senftenberg ATCC 43845 (light grey bars), *Salmonella* FUA 1934 (white bars), *Salmonella* FUA 1946 (dark grey bars), *Salmonella* FUA 1955 (medium grey bars), *E. faecium* NRRL B-2354 (hatched light grey bars), and *S. carnosus* FUA 2133 (hatched dark grey bars) inoculated onto beef jerky. Panel A; equilibrated to a_W 0.75 and treated at 5.7 MPa and 65 °C for 4, 8, and 15 min. Panel B; equilibrated to a_W 0.9 and treated at 5.7 MPa and 65 °C for 15 min. Panel C; equilibrated to a_W 0.75 and treated at 12.0 MPa and 65 °C for 4 and 15 min. Y-axis for all panels represents the treatment lethality calculated as $\log(N_0/N)$. n.d. indicates no data. * indicates no growth after enrichment. Data are shown as means ± standard deviation (n=3). Letters a-b denote significant differences among treatments in the same panel for the same strain ($P<0.05$). Letters x-z denote significant differences among strains in the same treatment group ($P<0.05$).

Figure 6. Effect of water-saturated supercritical N_2 on the reduction of *S.* Typhimurium ATCC 13311 (black bars), *Salmonella* FUA 1934 (white bars), and *S. carnosus* FUA 2133 (hatched dark grey bars) inoculated onto beef jerky and equilibrated to a_W 0.75 (A) or 0.9 (B), followed by treatment at 5.7 MPa and 65 °C for 15 min. Data are means ± standard deviation (n=3).

Table 1. List of bacterial strains and their source of isolation.

Strain	Source	Reference
Escherichia coli AW1.7	Slaughter facility	(Aslam et al., 2004)
E. coli AW1.7ΔpHR1	Derivative of AW1.7	(Pleitner et al., 2012)
E. coli K-12 MG1655, ATCC 700926	Laboratory	(Hauben et al., 1997)
E. coli 03-2832 O121:H19	Human	(Liu et al., 2015)
E. coli 05-6544 O26:H11	Human	(Liu et al., 2015)
E. coli C0283 O157:H7	Cattle	(Liu et al., 2015)
E. coli PARC 449 O145:NM	Unknown	(Liu et al., 2015)
E. coli 03-6430 O145:NM	Human	(Liu et al., 2015)
Enterococcus faecium NRRL B-2354	Dairy	(Kopit et al., 2014)
Pediococcus acidilactici FUA3072	Meat starter culture	(Tang et al., 2018)
S. Typhimurium ATCC 13311	Human	ATCC
S. Typhimurium ATCC 13311(pLHR)	Derivative of ATCC13311	(Seeras, 2017)
S. Typhimurium ATCC 13311(pRK767)	Derivative of ATCC13311	(Seeras, 2017)
S. Senftenberg ATCC 43845	Eggs	(Winter et al., 1946)
Salmonella FUA1934	Water treatment facility	This study
Salmonella FUA1946	Water treatment facility	This study
Salmonella FUA1955	Water treatment facility	This study
Staphylococcus carnosus FUA2133	Meat starter culture	(Tang et al., 2018)

Table 2. Lethality of treatment with water-saturated CO_2 at 5.7 MPa and 65 °C. Data shown are the reduction of cell counts [$\log(N_0/N)$] after desiccation, equilibration to a_w 0.75, followed by treatment for 1, 4, 8 or 15 min with water-saturated gas-phase CO_2 at 5.7 MPa and 65 °C. Data are shown as means ± standard deviation (n=3). Letters a-c denote significant differences among treatments for the same strain ($P<0.05$). Letters w-z denote significant differences among strains in the same treatment time ($P<0.05$).

Strains	**Drying only**	**1 min**	**4 min**	**8 min**	**15 min**
S. enterica ATCC13311	1.4 ± 0.6^{cxy}	6.1 ± 2.1^{abwx}	5.4 ± 0.6^{bxy}	5.8 ± 1.0^{bxy}	8.9 ± 0.1^{ax}
S. enterica ATCC43845	0.49 ± 0.27^{byz}	7.3 ± 1.5^{aw}	4.1 ± 3.5^{abxyz}	4.2 ± 1.9^{abxyz}	7.4 ± 2.1^{ax}
S. enterica FUA1934	0.58 ± 0.41^{byz}	1.7 ± 0.4^{abz}	1.3 ± 0.7^{byz}	2.5 ± 2.0^{abyz}	4.0 ± 1.6^{ayz}
S. enterica FUA1946	0.77 ± 0.60^{bxy}	2.8 ± 1.1^{abxyz}	1.9 ± 1.3^{abyz}	2.9 ± 1.6^{abyz}	5.6 ± 0.7^{axy}
S. enterica FUA1955	0.60 ± 0.21^{byz}	2.0 ± 0.6^{byz}	2.2 ± 2.1^{bxyz}	2.9 ± 2.6^{byz}	8.1 ± 0.7^{ax}
P. acidilactici FUA 3072	1.7 ± 0.3^{bx}	5.4 ± 2.1^{awxy}	6.4 ± 0.7^{ax}	7.8 ± 0.2^{ax}	7.9 ± 0.1^{ax}
E. faecium NRRL B-2354	0.21 ± 0.01^{bz}	0.9 ± 2.1^{abz}	1.5 ± 0.4^{abyz}	1.8 ± 0.5^{abyz}	2.4 ± 1.3^{ayz}
S. carnosus FUA 2133	0.04 ± 0.18^{z}	0.33 ± 0.33^{z}	0.4 ± 0.1^{z}	0.5 ± 0.4^{z}	1.5 ± 1.4^{z}

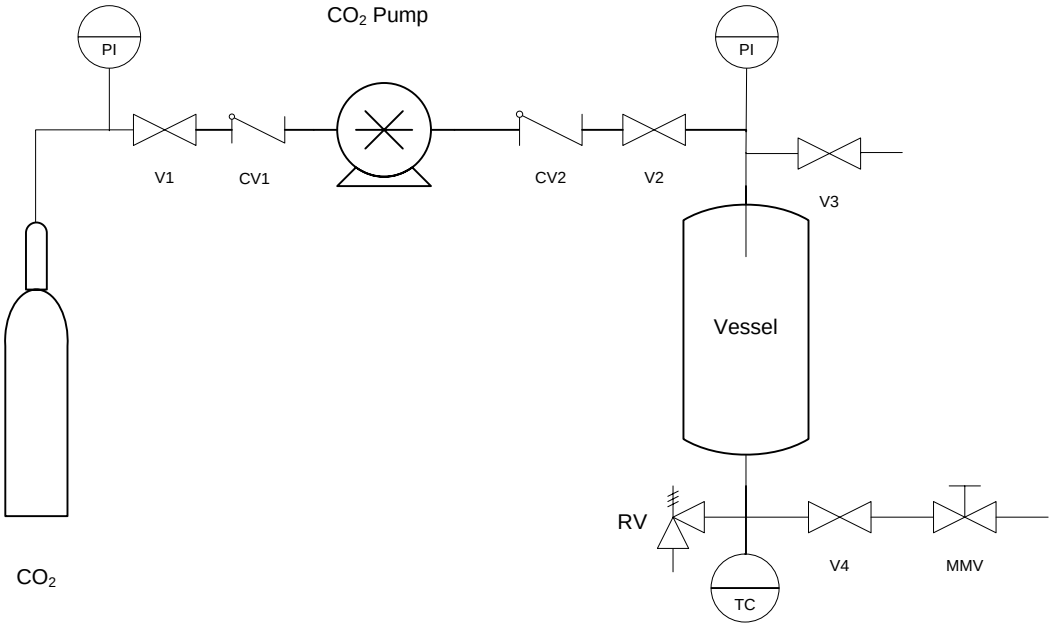
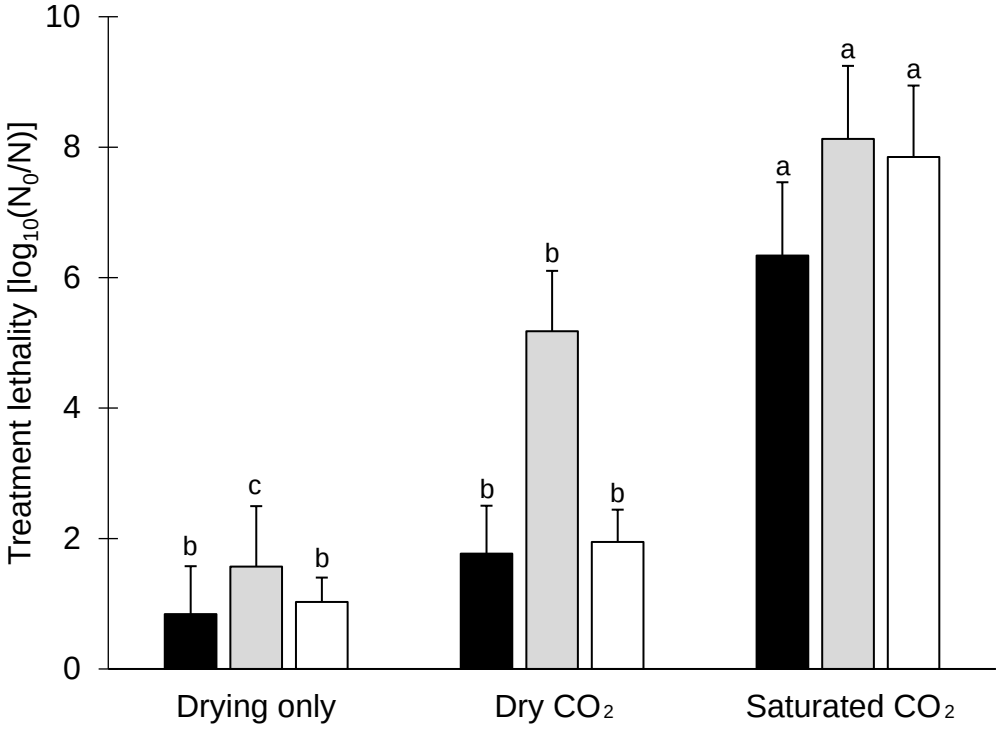


Figure 1



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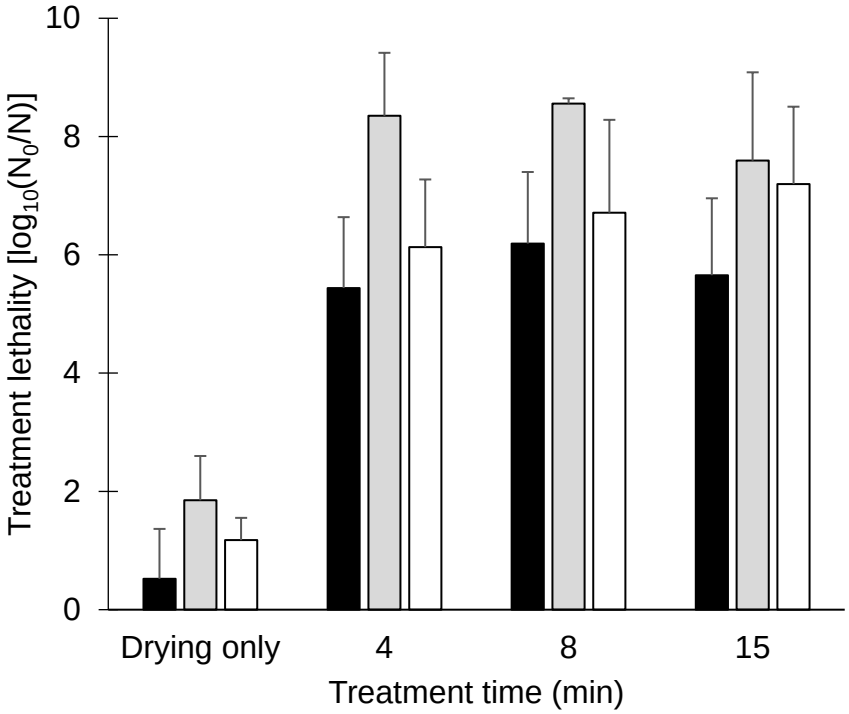

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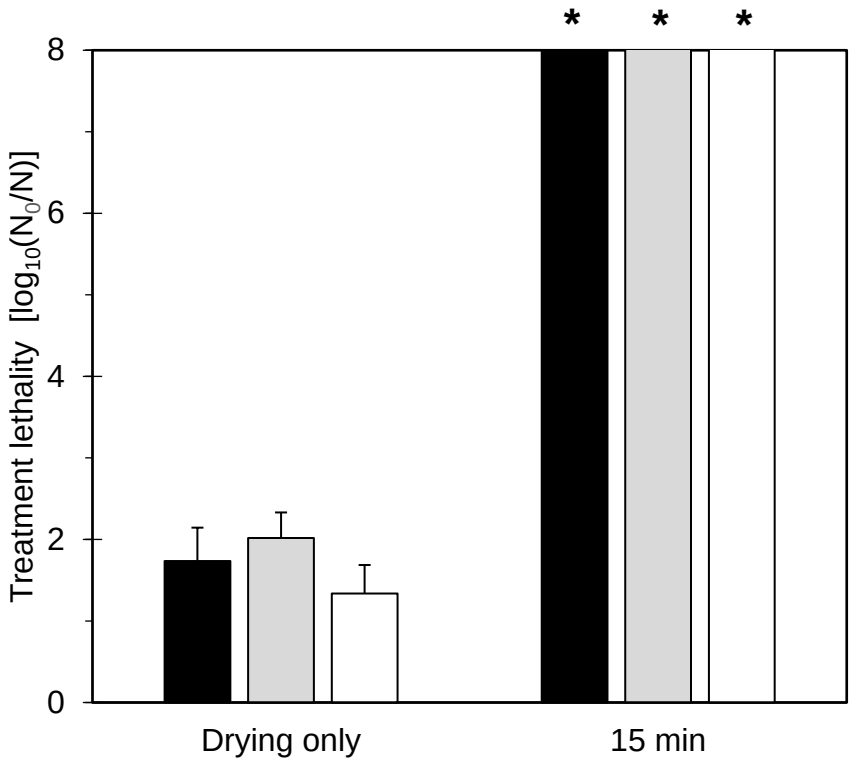

Figure 4. Effect of saturated CO_2 at 5.7 MPa and 65 °C for 15 min on the reduction of *E. coli* AW1.7 (black bars), *E. coli* MG1655 (grey bars), and the STEC cocktail (white bars) inoculated onto beef jerky and equilibrated to a_W 0.75. Y-axis was limited to detection limit. * indicates no growth after enrichment. Data are shown as means ± standard deviation (n=3).

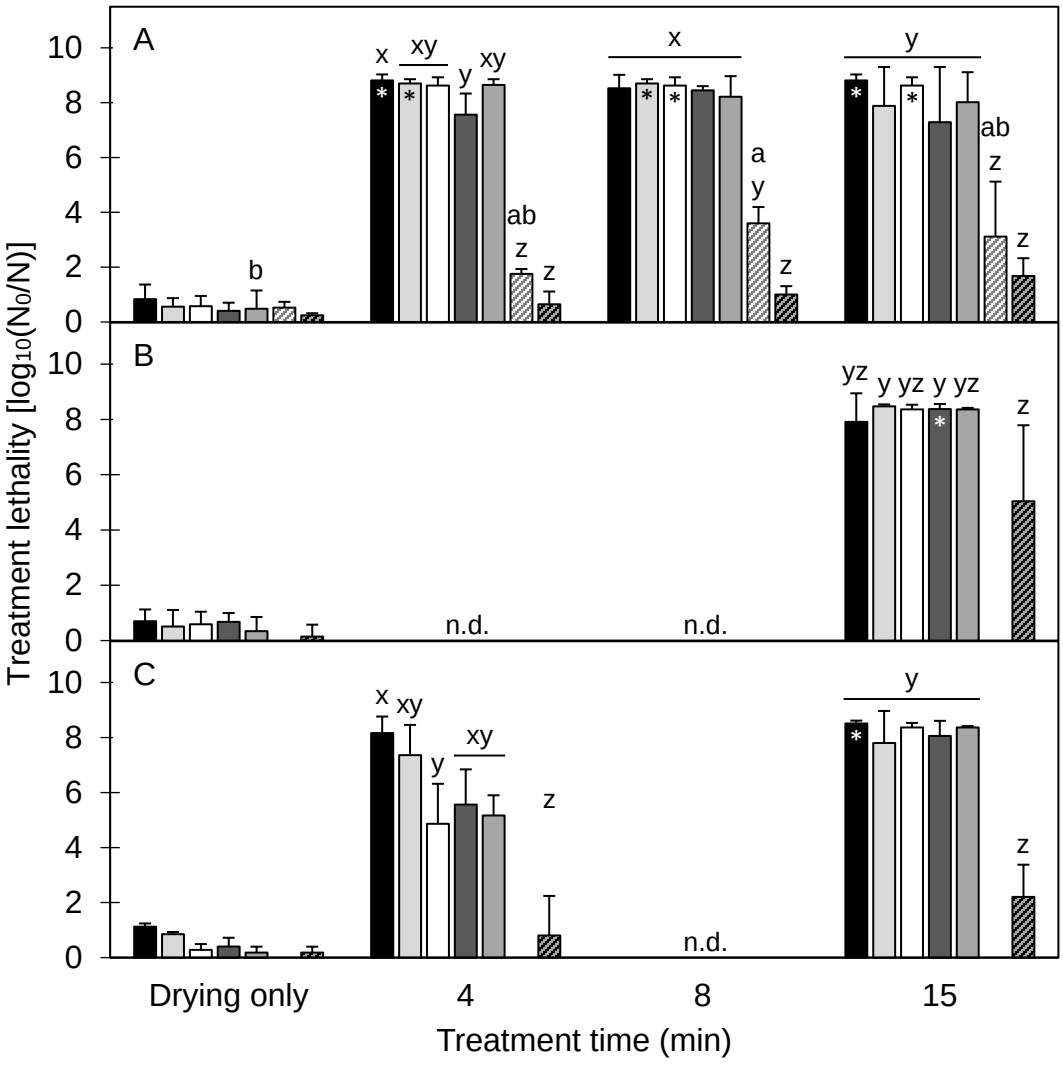

Figure 5. Reduction of *S.* Typhimurium ATCC 13311 (black bars), *S.* Senftenberg ATCC 43845 (light grey bars), *Salmonella* FUA 1934 (white bars), *Salmonella* FUA 1946 (dark grey bars), *Salmonella* FUA 1955 (medium grey bars), *E. faecium* NRRL B-2354 (hatched light grey bars), and *S. carnosus* FUA 2133 (hatched dark grey bars) inoculated onto beef jerky. Panel A; equilibrated to a_W 0.75 and treated at 5.7 MPa and 65 °C for 4, 8, and 15 min. Panel B; equilibrated to a_W 0.9 and treated at 5.7 MPa and 65 °C for 15 min. Panel C; equilibrated to a_W 0.75 and treated at 12.0 MPa and 65 °C for 4 and 15 min. Y-axis for all panels represents the treatment lethality calculated as $\log(N_0/N)$. n.d. indicates no data. * indicates no growth after enrichment. Data are shown as means ± standard deviation (n=3). Letters a-b denote significant differences among treatments in the same panel for the same strain ($P<0.05$). Letters x-z denote significant differences among strains in the same treatment group ($P<0.05$).

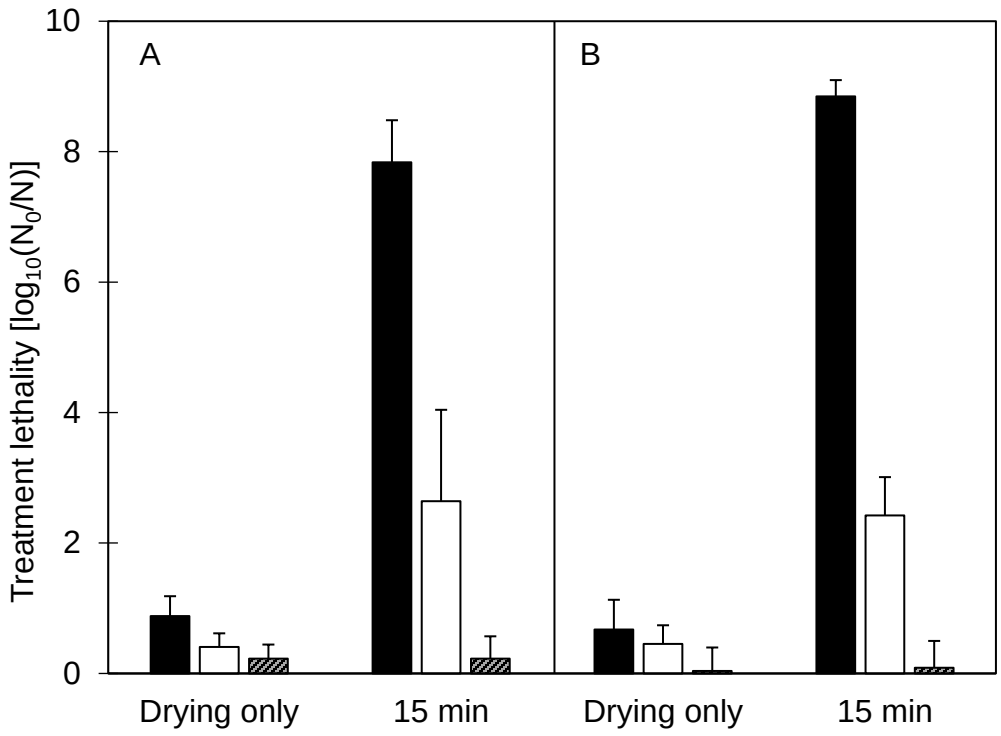

Figure 6. Effect of water-saturated supercritical N_2 on the reduction of *S.* Typhimurium ATCC 13311 (black bars), *Salmonella* FUA 1934 (white bars), and *S. carnosus* FUA 2133 (hatched dark grey bars) inoculated onto beef jerky and equilibration to a_w 0.75 (A) or 0.9 (B), followed by treatment at 5.7 MPa and 65 °C for 15 min. Data are shown as means ± standard deviation (n=3).