Campylobacter jejuni and Chicken Livers: Real World Problems in Need of Real World Solutions

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Outbreaks Caused by *Campylobacter jejuni* Contaminated Chicken Livers

Multi-year, multi-nation outbreaks of *C. jejuni* mediated disease have occurred in recent years with chicken livers implicated as the food vector for the outbreaks

Chicken livers frequently contaminated with *Campylobacter* with reported prevalence percentages ranging between 31 and 100%

Contamination levels as high as 2×10^4 cells/ml in chicken livers reported

Many of the outbreaks have resulted from the consumption of pâté products made from chicken livers

It has been suggested that consumer preference for maintaining a pink color in the chicken liver meat or pâté results in preferential undercooking of the product allowing for survival of *Campylobacter* cells

We are therefore faced with two challenges:

1) Product contamination levels by the disease causing agent that approach 100%
2) Consumer's willful persistent to not properly cook the food product

Therefore we need a method to produce an almost sterile chicken liver product

Application of Available Intervention Techniques to Reduce *C. jejuni* Numbers in Chicken Livers

Previous research has observed *Campylobacter* contamination levels at levels as high as 20,000 cells/ml or roughly 400,000 cells/ liver. Five log reduction in *Campylobacter* numbers would be ideal.

Chicken livers purchased from retail source and then irradiated to sterility (20 kGy). Stored frozen until used in experiments.

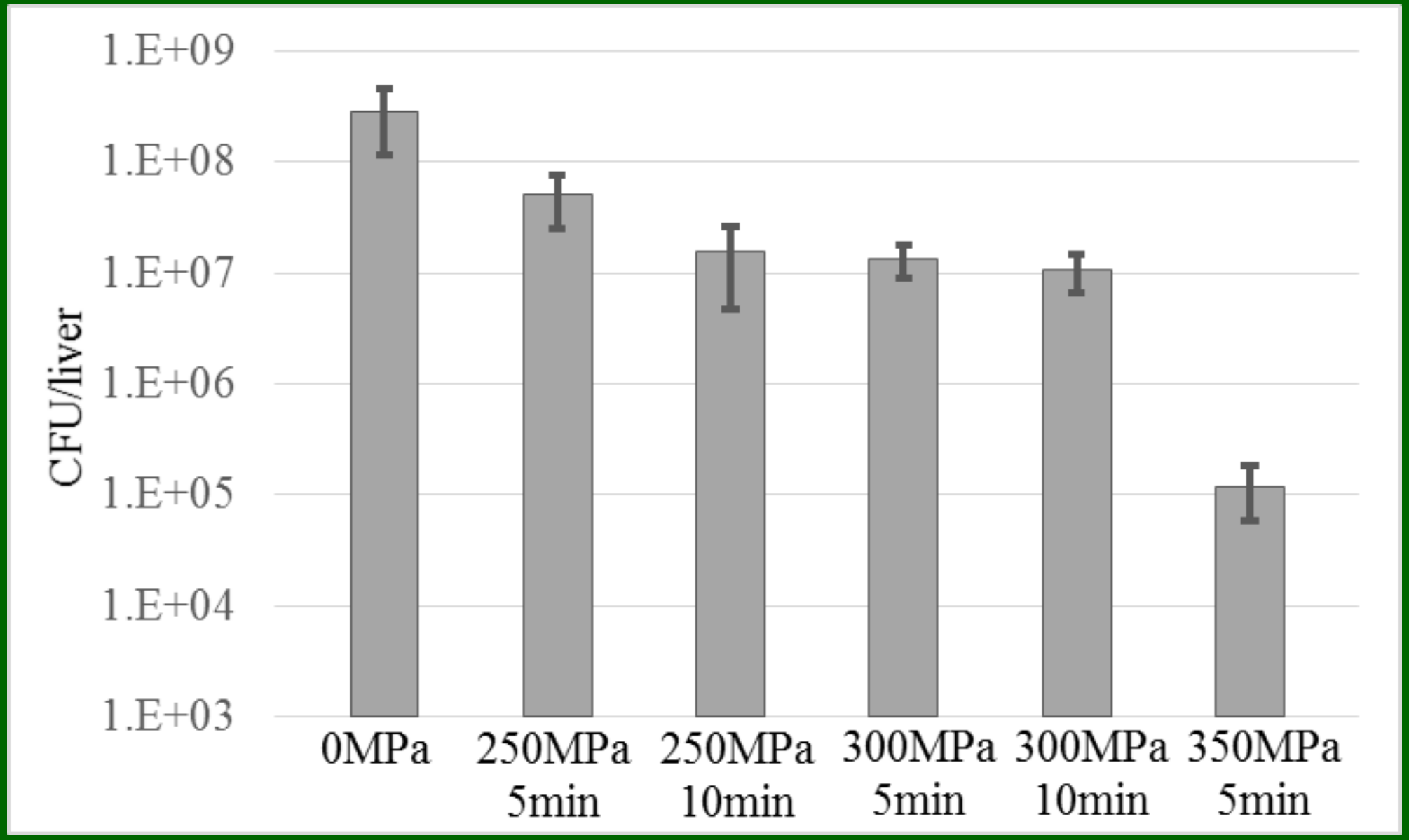
Livers experimentally contaminated with a cocktail of the 7 *C. jejuni* FSIS outbreak strains. The concentration of the individual strains normalized by OD_{600} measurement prior to mixing.

High- hydrostatic pressure treatment: Pressure treatment ranging from 250 to 350 MPa applied to experimentally contaminated chicken livers at refrigeration temperatures (4°C)

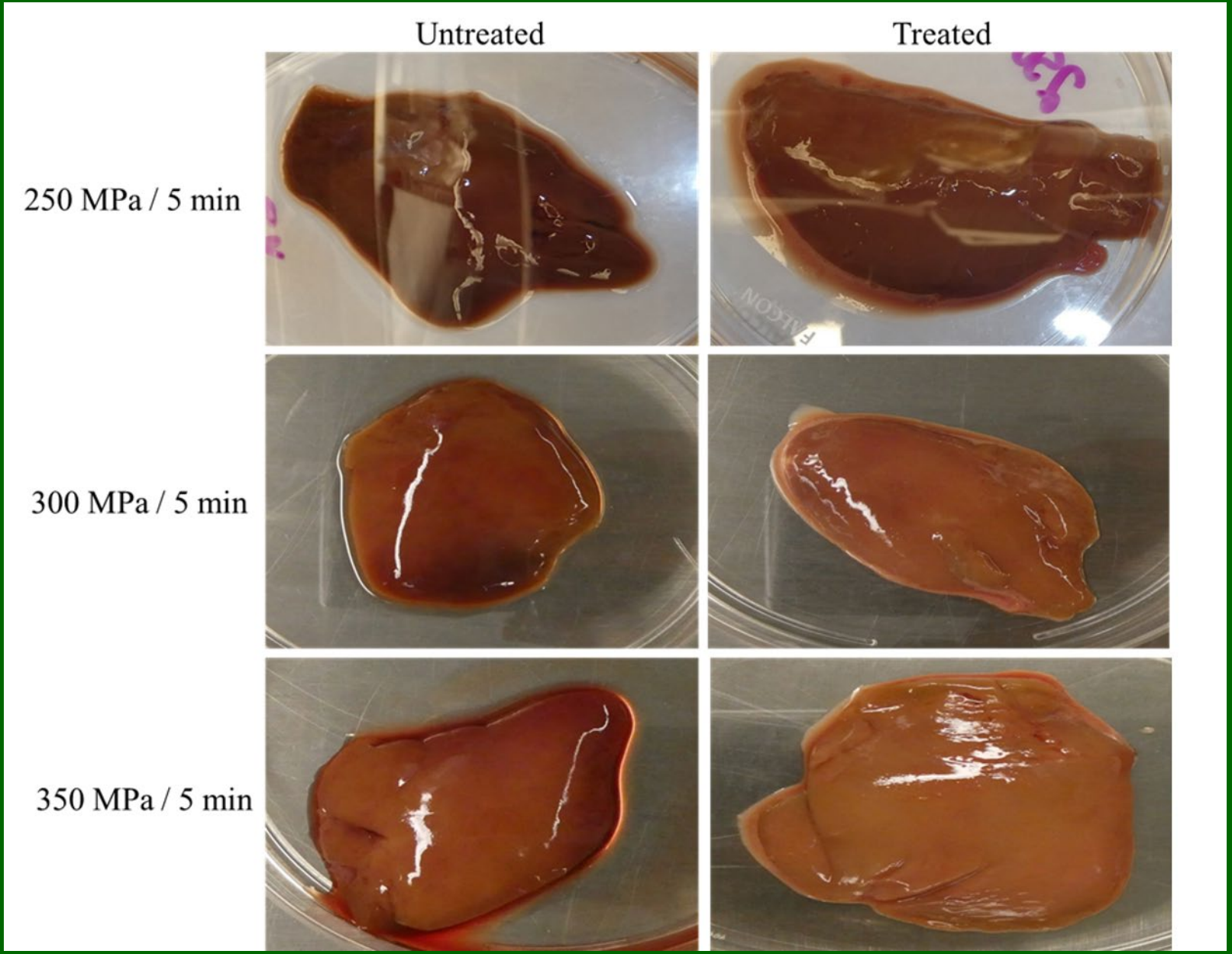
ɣ-Irradiation treatment: A range of irradiation doses 0 - 2.0 kGy applied to the experimentally contaminated chicken livers at refrigeration temperatures (4°C)

The resulting treated and control livers from each experiment were homogenized, serially diluted and directly plated to determine the surviving *C. jejuni* numbers.

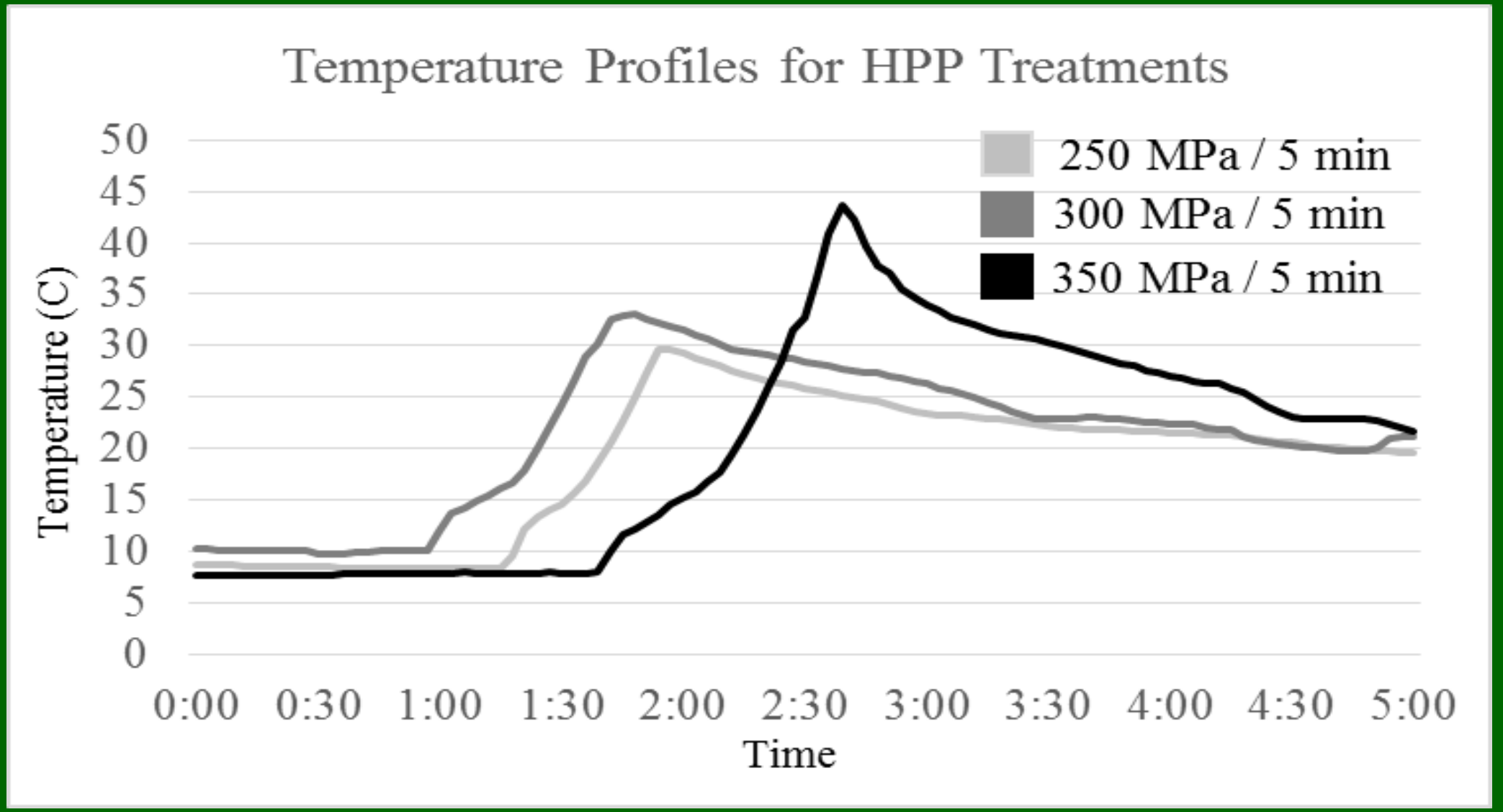
Different High Pressure Treatments for the Reduction of *C. jejuni* in Chicken livers



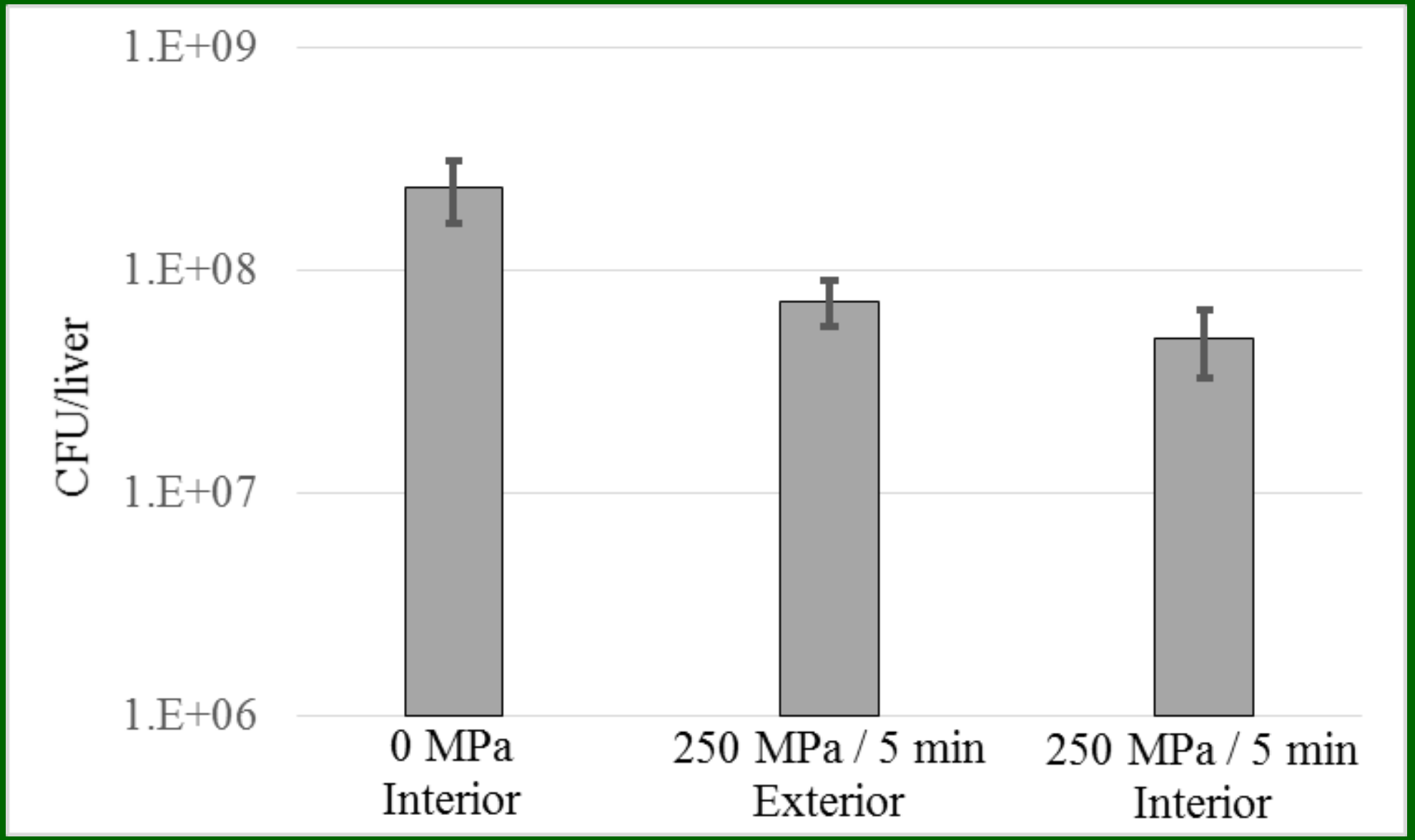
The Effect of High Pressure on Chicken Liver Appearances



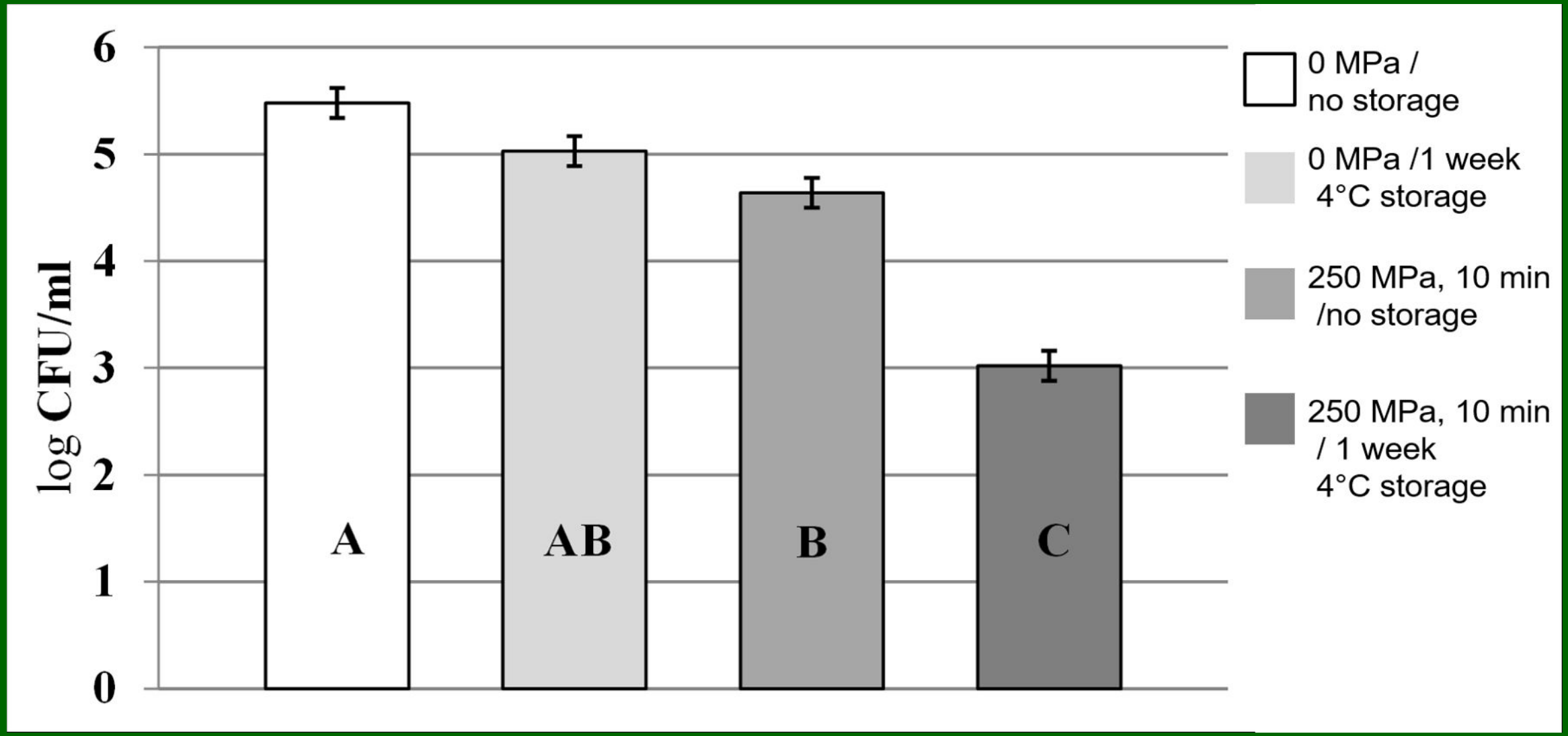
Temperature Effects Produced by High Pressure Treatment



High Pressure Treatment of *C. jejuni* on the Interior or Exterior of Chicken Livers

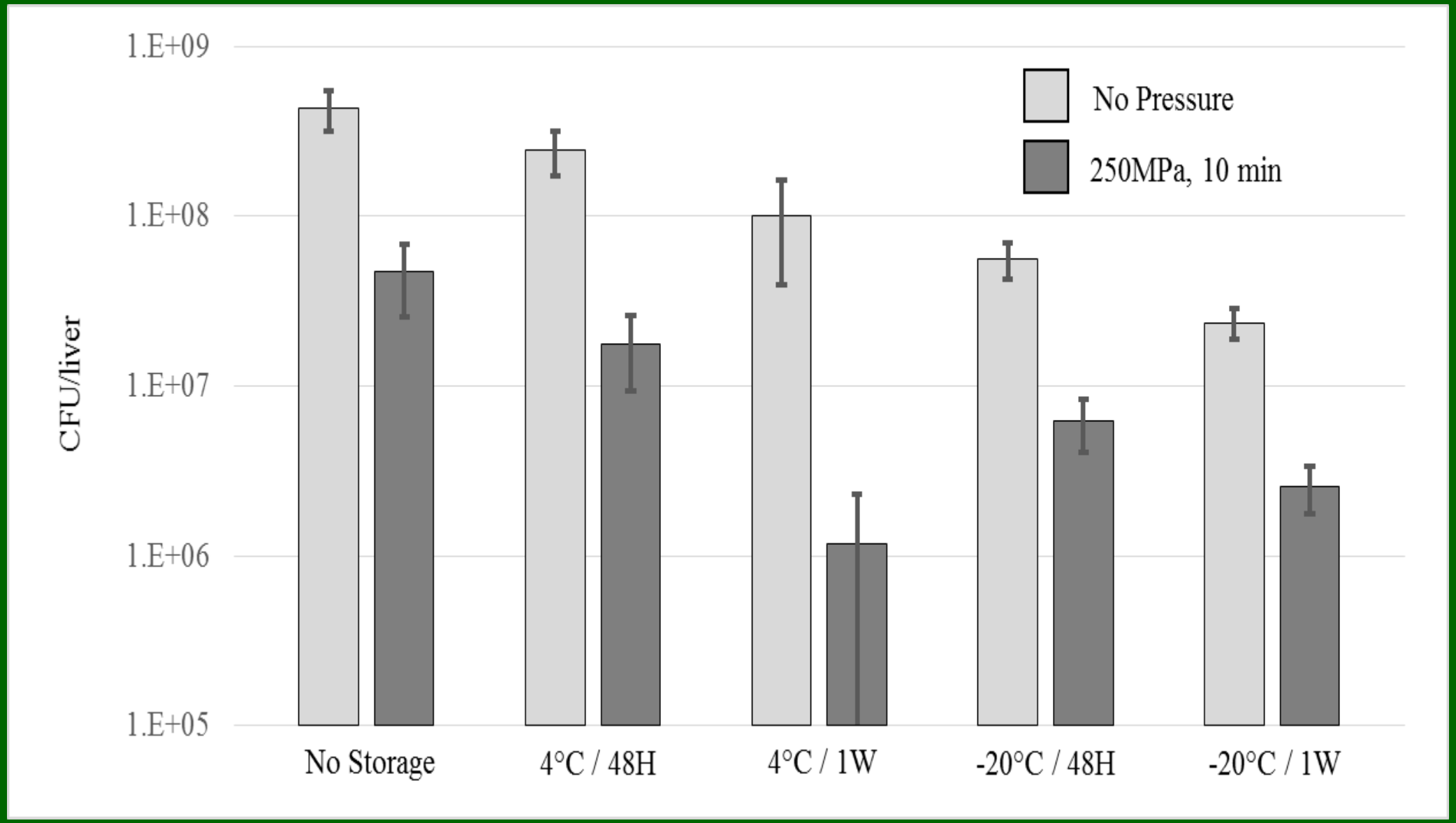


Previous Study: High Pressure Treatment Followed by Cold Storage

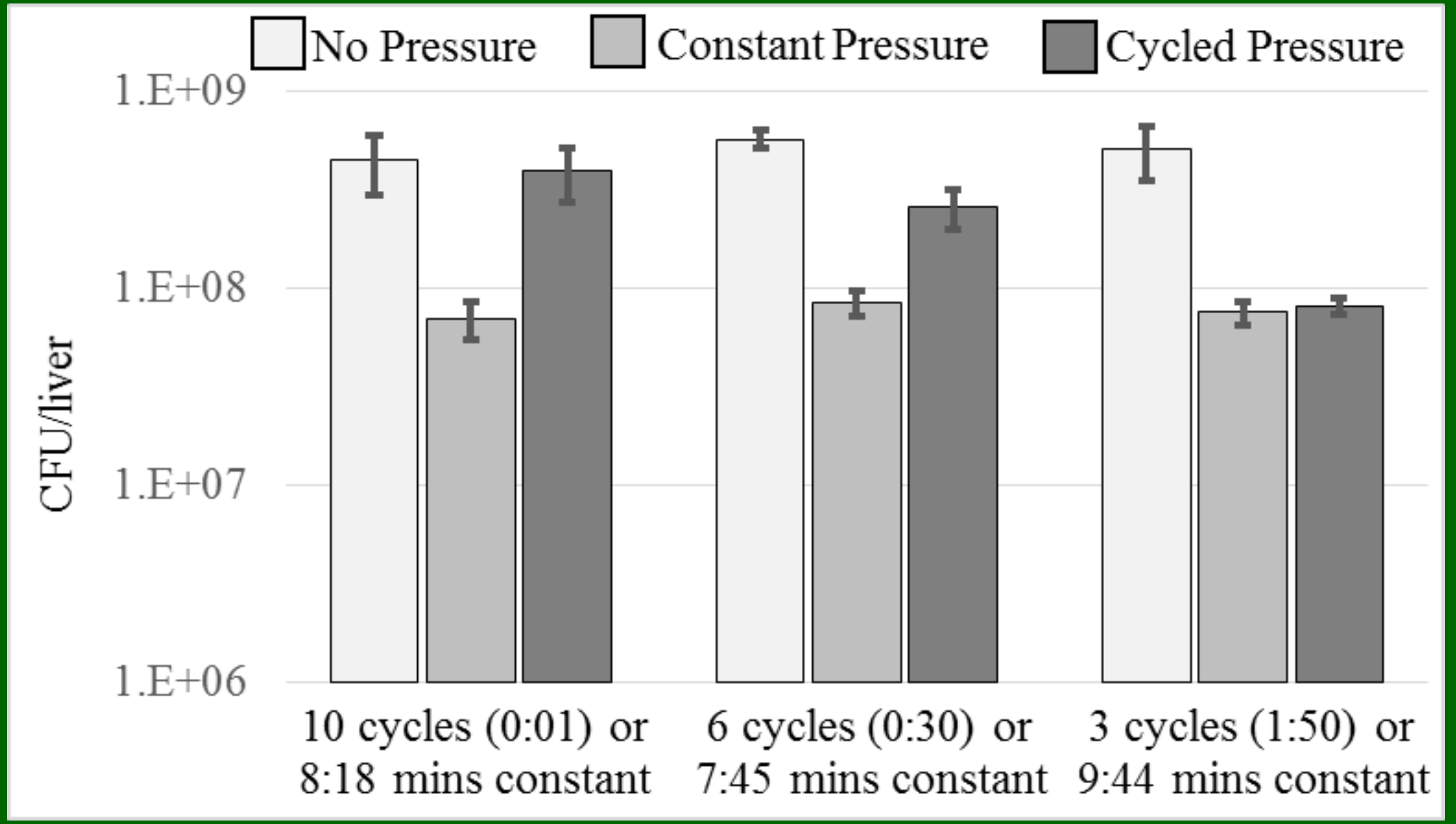


Gunther NW, 4th, Sites J, Sommers C. The effects of high-pressure treatments on *Camplylobacter jejuni* in ground turkey products containing polyphosphate additives. Poultry Sci (2015) 94:2297-302

High Pressure Treatment Followed by Cold Storage



Constant High Pressure Compared to Cycled High Pressure Treatments



Overview of High Pressure Treatment on *C. jejuni* in Chicken Livers

High pressure treatment of 350 MPa for 5 minutes produces an average reduction in *C. jejuni* numbers of 3.4 logs, but results in color changes in the product

High pressure treatment of 250 MPa for 10 minutes minimizes color changes but only produces an average reduction of 1.3 logs

The effectiveness of high pressure treatments in reducing *C. jejuni* is not impacted by the bacteria being located internal or external to the livers

Cold storage can produce an addition average reduction of up to 1.3 logs

- Better reductions observed in previous HPP/cold storage experiments in ground turkey
- iron levels instrumental in resuscitating *C. jejuni* sub-lethally injured by HPP

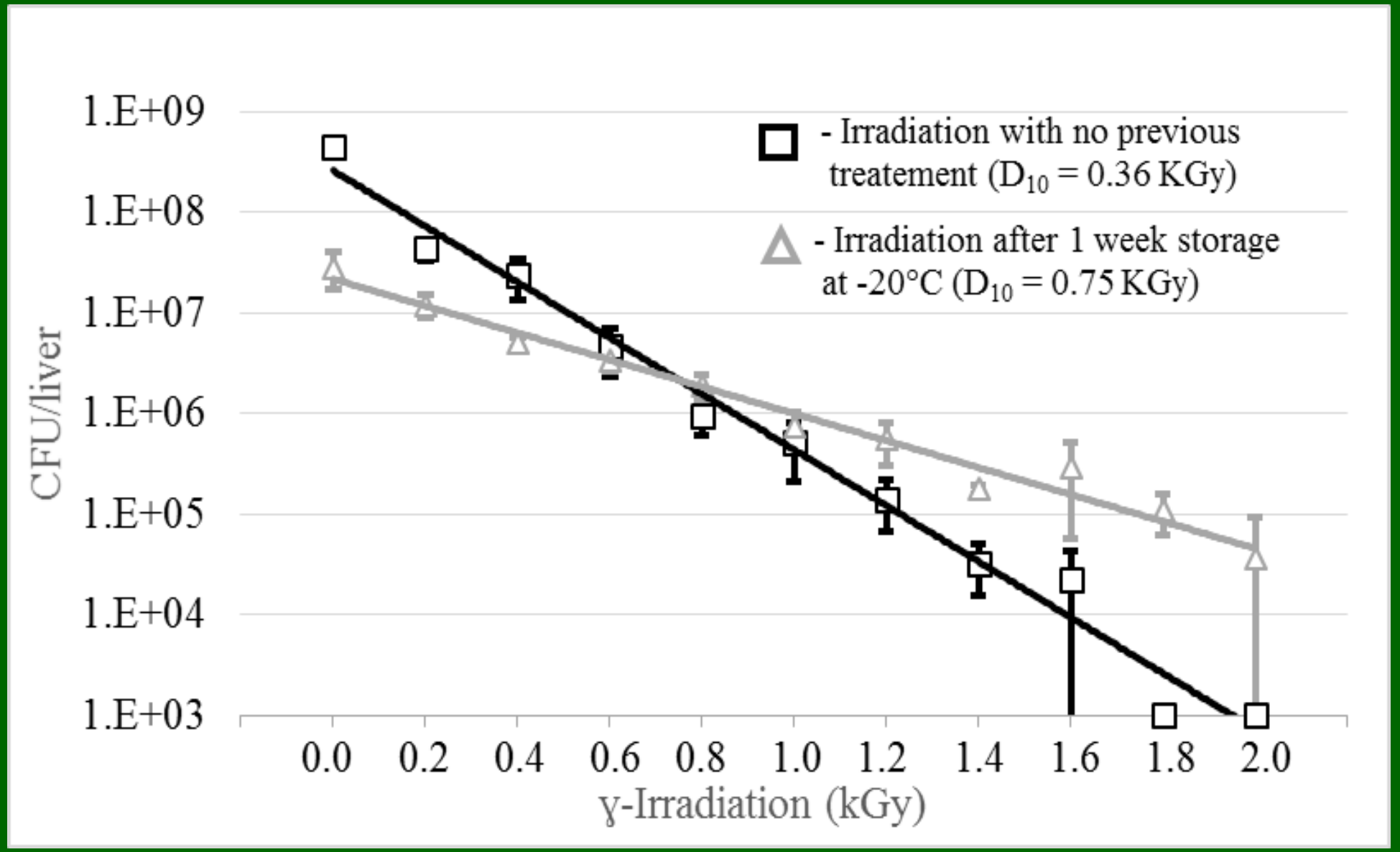
Cycled application of high pressure did not produce greater reductions in *C. jejuni* numbers compared to constant pressure application.

High pressure applied to livers marinade with anti-microbial flavor compounds.

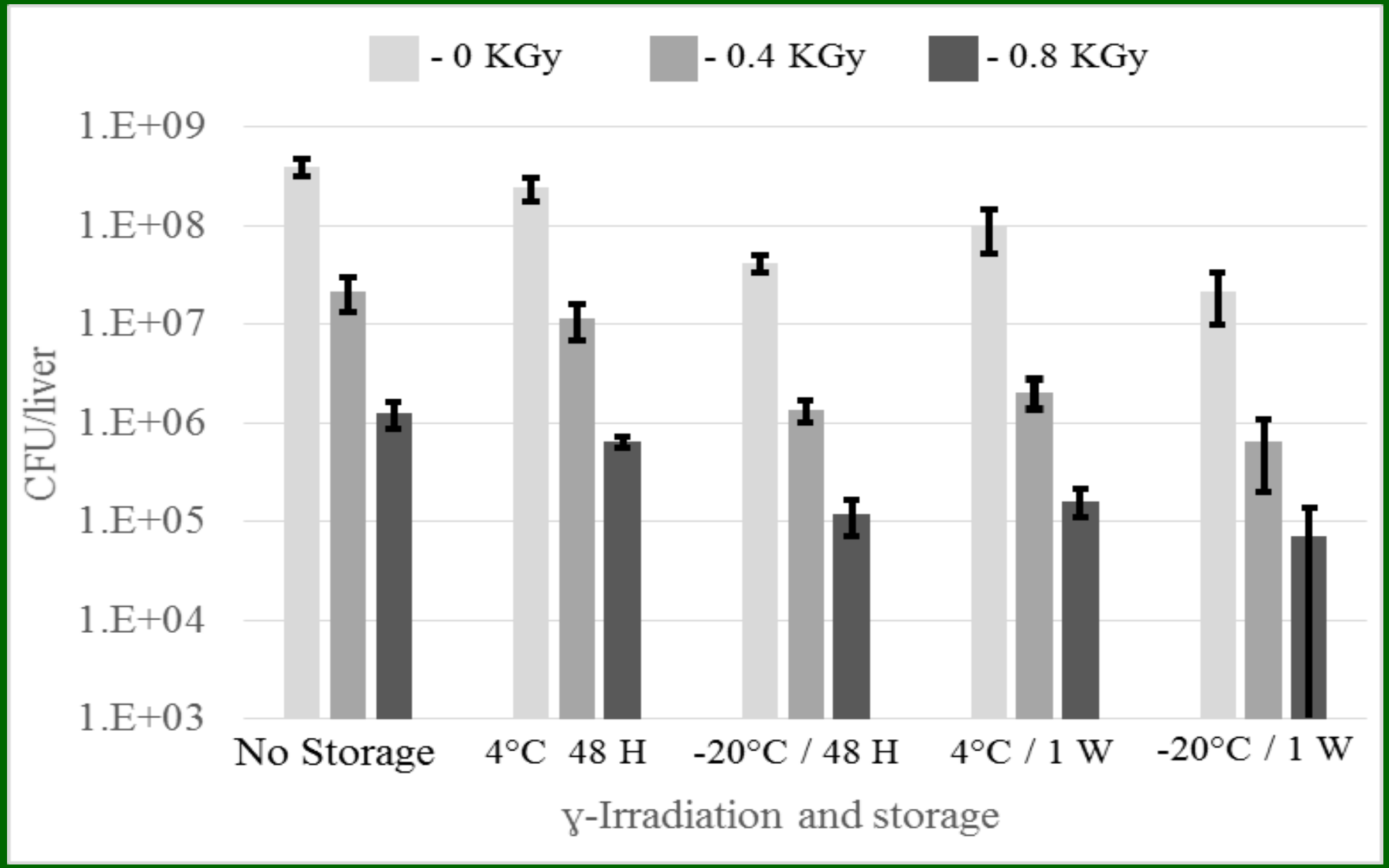
Common pâté flavors: onion, garlic, bay leaves, thyme, various alcohols

Less common pâté flavors: cayenne, nutmeg, rosemary, sage, olive oil

Irradiation of Chicken Livers Contaminated with *C. jejuni*



Irradiation Followed by Cold Storage



Overview of ɣ-Irradiation on *C. jejuni* in Chicken Livers

Livers contaminated with C. jejuni and without frozen storage (-20°C) directly prior to irradiation had a D_{10} value of 0.361 kGy

1.8 kGy of irradiation produced >5 log average reduction in *C. jejuni* numbers in the unfrozen livers

Livers contaminated with *C. jejuni* and with 1 week frozen storage (-20°C) directly prior to irradiation had a D_{10} value of 0.748 kGy

1.8 kGy of irradiation produced 3-4 log average reduction in *C. jejuni* numbers in the frozen livers

Cold storage of livers post-irradiation increased *C. jejuni* average reductions over irradiation alone

0.8 kGy followed by storage at -20°C for 1 week produced *C. jejuni* average reduction of 3.8 logs

0.8 kGy with storage at -20°C for 48H produced only a slightly decreased average reduction, 3.5 logs

Irradiation appears to be a method capable of producing livers with extremely reduced *C. jejuni* levels

3 obvious issues:

1) Cost
2) Effects on liver quality
3) Consumer acceptance of irradiated product

Whole Genome Sequencing of Outbreak Strains

Seven strains isolated from chicken livers implicated in disease outbreaks:

FSIS 135 (*C. jejuni*)
FSIS 136 (*C. jejuni*)
FSIS 137 (*C. jejuni*)
FSIS 138 (*C. jejuni*)
FSIS 139 (*C. jejuni*)
FSIS 140 (*C. jejuni*)
FSIS 141 (*C. jejuni*)

Eight strains isolated from human patients sickened in outbreaks:

CDC 9503(*C. jejuni*)
CDC 9511 (*C. jejuni*)
CDC 9505 (*C. jejuni*)
CDC 0278 (*C. coli*)
CDC 9509 (*C. jejuni*)
CDC 0279 (*C. jejuni*)
CDC 9510 (*C. jejuni*)
CDC 0281 (C. jejuni)

PacBio-based whole genome sequencing followed by whole genome comparisons to identify potential resistance or enhanced survival profiles. The comparisons will also include 6 *C. jejuni* (2 chicken/4 human) and 6 *C. coli* (6 chicken) strains previously sequenced by our group.

Previously sequenced *Campylobacter* include strains that we have identified to possess specific resistance traits

RM 3194 – Biomarker for UV light resistance (plus type VI secretion system)
RM 1285 – Biomarker for high pressure resistance
RM 1246 ERRC – non-specific quaternary ammonium compound resistance

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