Difficult to Inactivate Microorganisms

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Solutions *for* TODAY
Planning *for* TOMORROW

May 6-9, 2019 | Rosemont, IL
Donald E. Stephens Convention Center

Outline

Set the scene

- Food processing perspective

Food technologies

- Balance between safety and quality
- Examples

Challenges

A Balancing Act

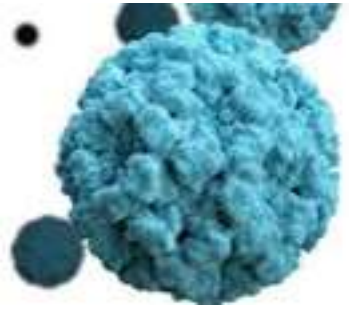
Food Safety Balance

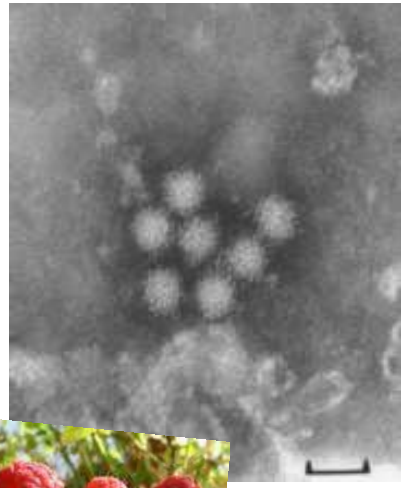
Need to destroy
Pathogens
Spoilage Organisms
Enzymes

vs

Optimise
Flavour
Texture
Colour
Nutritional quality

Outbreaks





- Ingredients and finished products are affected
- Global trade that impact multiple countries
  - HAV frozen berries from Canada, Serbia and Poland with cases in Italy
  - NoV in frozen strawberries from China affecting 12,000 in Germany
  - 2018 Winter Olympics
- Effective controls measures throughout food chain

15/03/2019	2019.1003	Germany	norovirus (GII /25g) in frozen red currants from Poland	fruits and vegetables
22/02/2019	2019.0686	France	foodborne outbreak suspected to be caused by norovirus (GI and GII /2g) in live oysters from France	bivalve molluscs and products thereof
22/02/2019	2019.0691	Italy	foodborne outbreak suspected to be caused by norovirus (GII /2g) in live oysters (Crassostrea gigas) from France	bivalve molluscs and products thereof
13/02/2019	2019.0554	Netherlands	norovirus (GI and GII /2g) in live oysters (Crassostrea gigas) to be purified from Portugal	bivalve molluscs and products thereof

Hepatitis A sickens 17 in Denmark with link to dates

By Joseph James Whitworth

Effectiveness of Control Measures

[1] ICMSF conceptual equation Microbiological testing in Food Safety Management, ICMSF (International Commission on Microbiological Specifications for foods) (2002); Book 7

$$H_0 - \Sigma R_{A,B,C} + \Sigma I_{A,B,C} \leq FSO$$

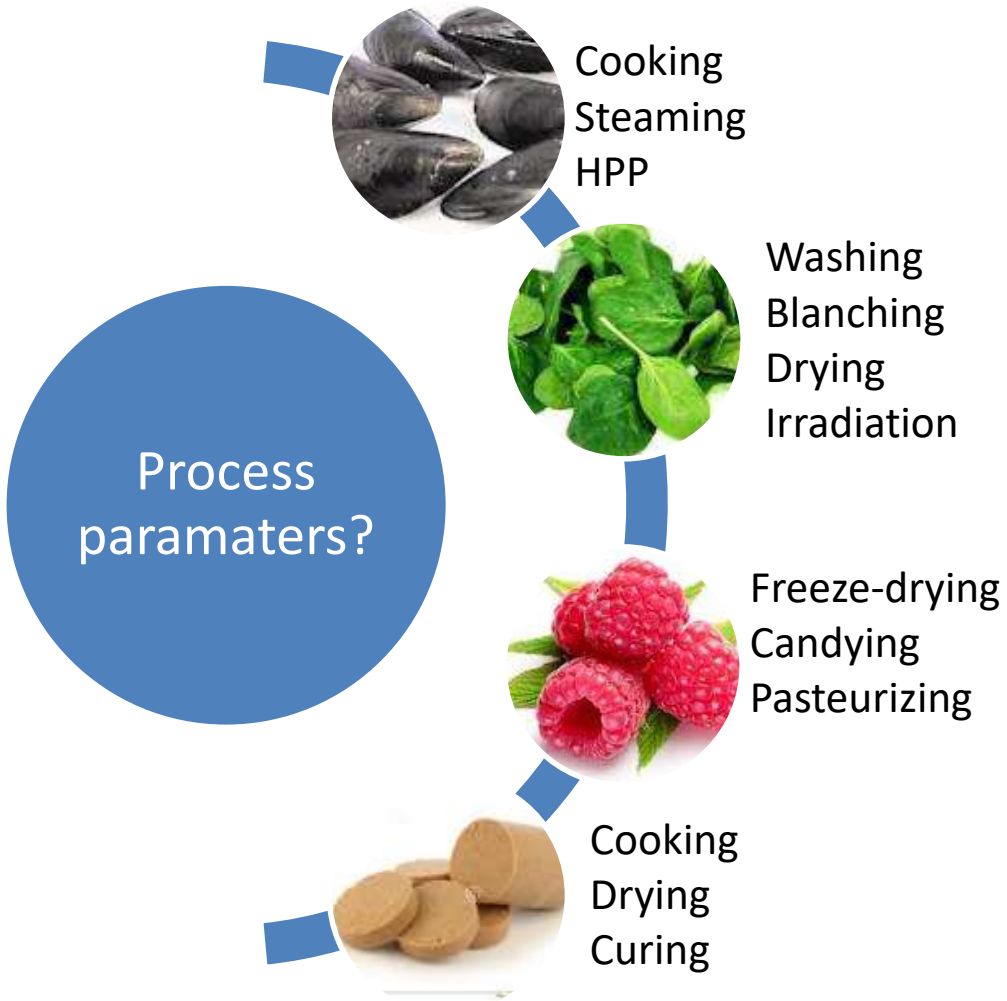
Initial load at primary production

Reduction (Supplier and Factory)

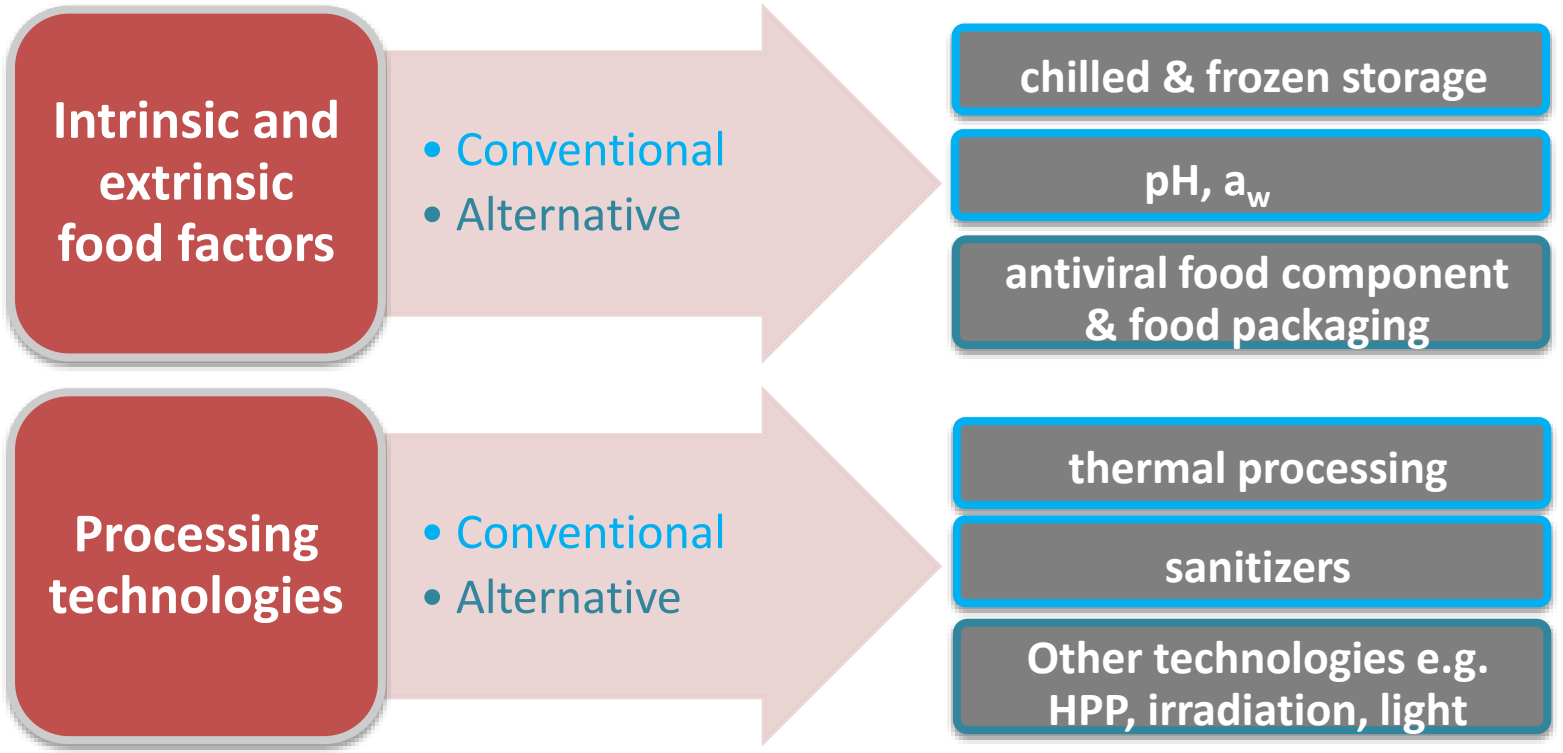
Increase (Growth, Recontamination)

Performance Objective or Food Safety Objective

Which Matrices-Process Combinations?

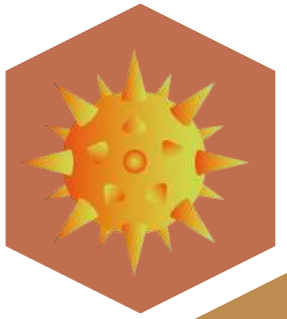


Control Measures



Virus Inactivation Studies: Challenges

Pathogen *versus* surrogate



NoV

MNV (Murine Norovirus)
FCV (Feline calicivirus)
TV (Tulane virus)
Bacteriophages, e.g. MS2

HAV

HAV HM-175

HEV

HEV genotype 3
strain 47832c

Laboratory scale *versus* pilot scale
RT-qPCR *versus* infectivity assay
Cell culture media *versus* food matrix

AMERICAN SOCIETY FOR MICROBIOLOGY | Applied and Environmental Microbiology

Thermal Stability of Hepatitis E Virus as Estimated by a Cell Culture Method

Reimar Johne,[a] Eva Trojnar,[a] Matthias Filter,[a] Jörg Hofmann[b]

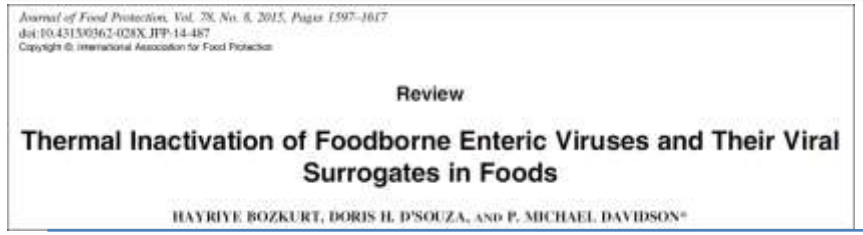
Federal Institute for Risk Assessment, Berlin, Germany[a]; Institute of Medical Virology, Charité Medical School, Berlin, Germany[b]

Johne et al., 2016

Thermal Processing

Boiling water (for min 60s) effectively (>4 log_{10} reduction) inactivates viruses (enteroviruses, HRV, huNoV, HAV and HEV) that are transmitted by contaminated water (CDC 2009)

HAV


Journal of Food Protection, Vol. 78, No. 8, 2015, Pages 1597–1617
doi:10.4315/0362-028X.JFP-14-487

Review

Thermal Inactivation of Foodborne Enteric Viruses and Their Viral Surrogates in Foods

HAYRIYE BOZKURT, DORIS H. D'SOUZA, AND P. MICHAEL DAVIDSON*

Control measures	Matrix	Virus	Log_{10} reduction	Reference
72°C, <0.3 min	Cell culture medium	HAV	1	Hewitt, 2009
72°C, 0.88 min	Cell culture medium	HAV	1	Bozkurt, 2014
72°C, 0.91 min	Spinach	HAV	1	Bozkurt, 2015
72°C, 1.07 min	Mussels	HAV	1	Bozkurt, 2014
85°C, 1 min	Strawberry mashes (28° Brix)	HAV	1	Deboosere, 2004
85°C, 5 min	Strawberry mashes (52° Brix)	HAV	1	Deboosere, 2004
80°C, 20 min	Freeze-dried berries	HAV	<2	Butot, 2009

NoV and Its Surrogates

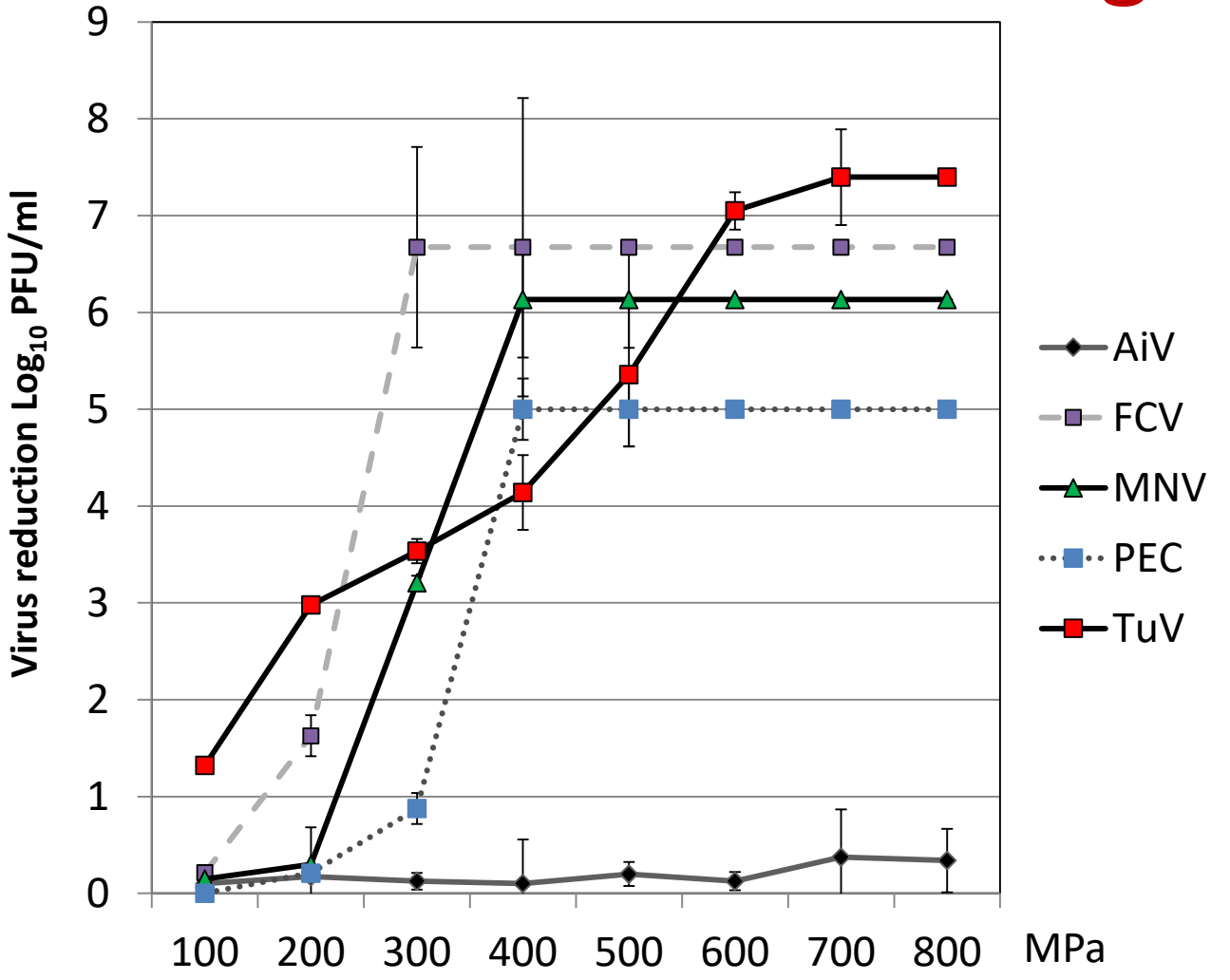
NoV

thermal processsing

Control measures	Matrix	Virus	Log_{10} reduction	Reference
72°C, 1 min	Water	MNV	>3.5	Hewitt *et al* 2009
80°C, 1 min	Spinach	MNV	≥ 2.4	Baert *et al.* 2008
75°C, 0.25 min	Raspberry puree	MNV	2.8	Baert *et al.* 2008
95°C, 2.5 min	Basil	FCV	> 4	Butot *et al.* 2009
60°C, 15 min	Stool	HuNoV	>5	Ettayebi *et al.*, 2016

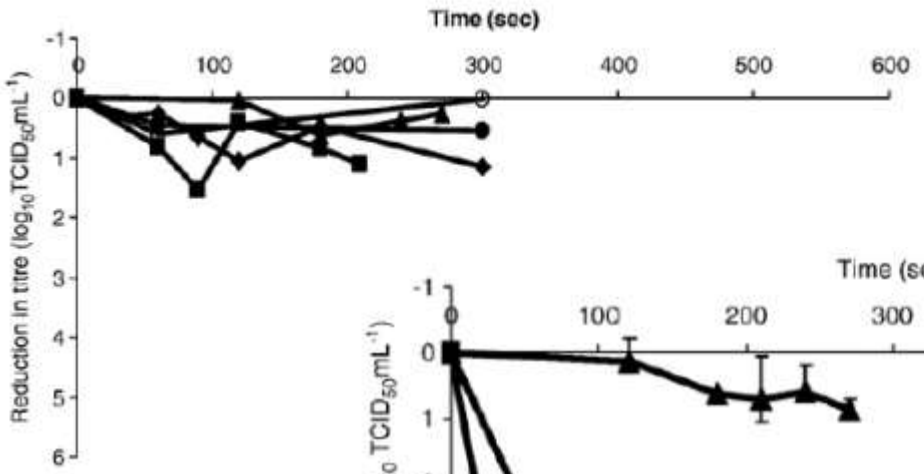
- How will HuNoV inactivation data compare with the different surrogates?

HPP Inactivation of Surrogates

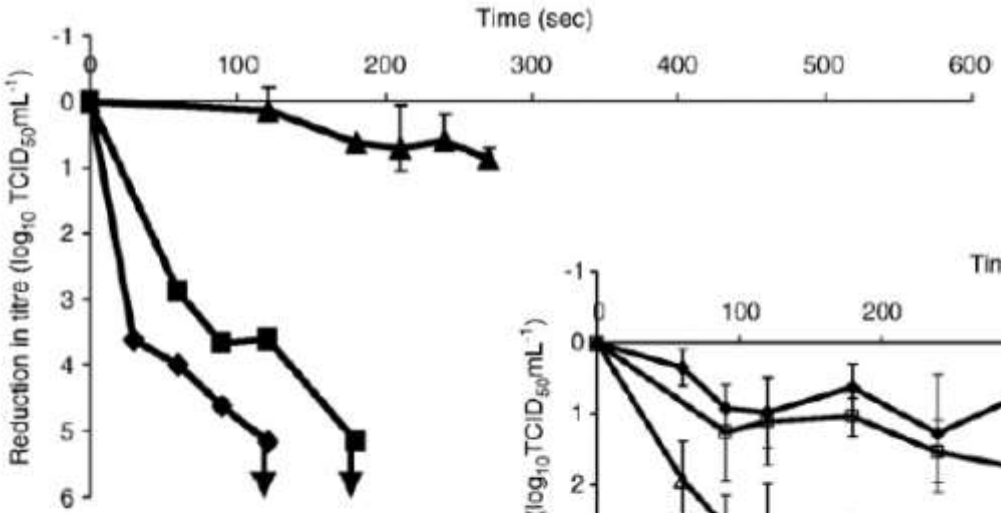


Cromeans *et al.,* 2013 AEM

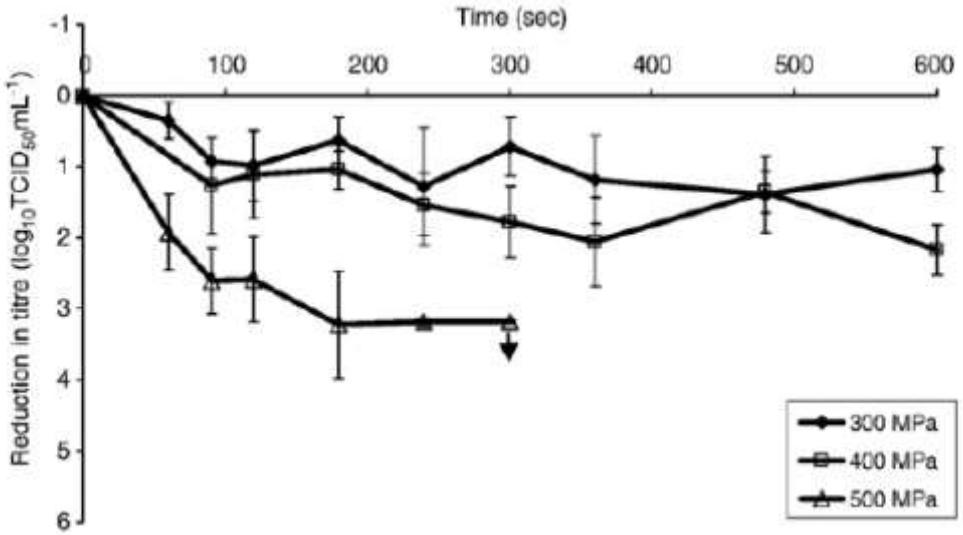
High Pressure Processing and Viruses



Poliovirus



Feline calicivirus



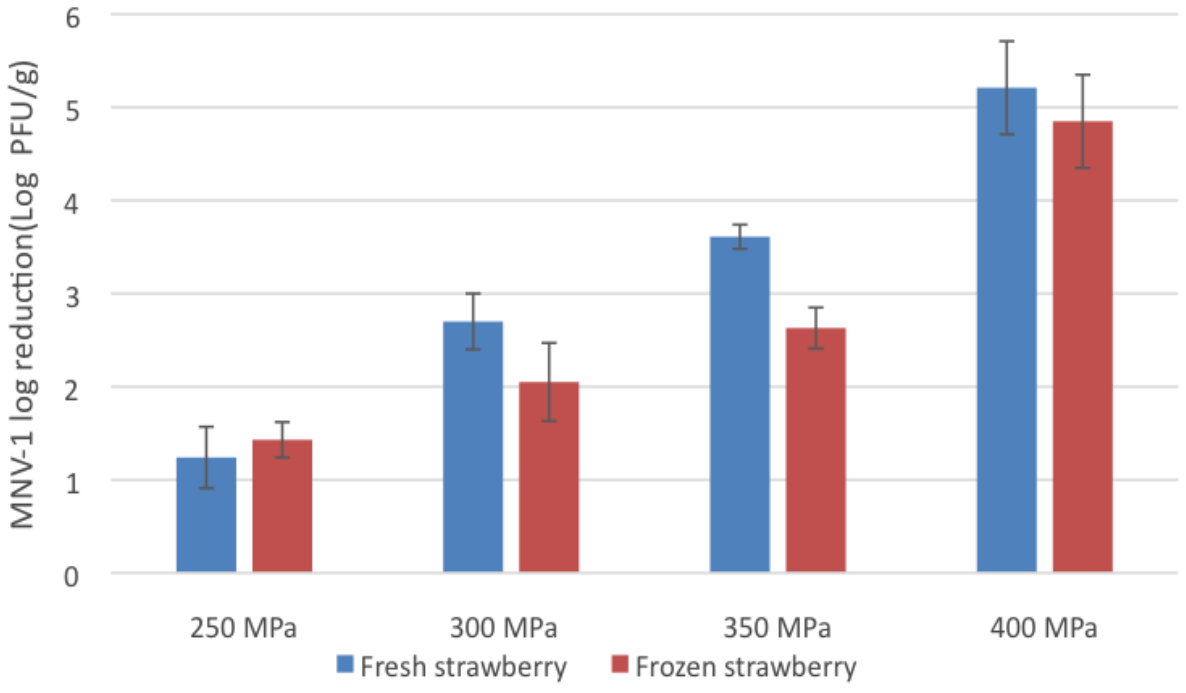
Hepatitis A virus

Grove *et al*., 2008
J. Food Protection

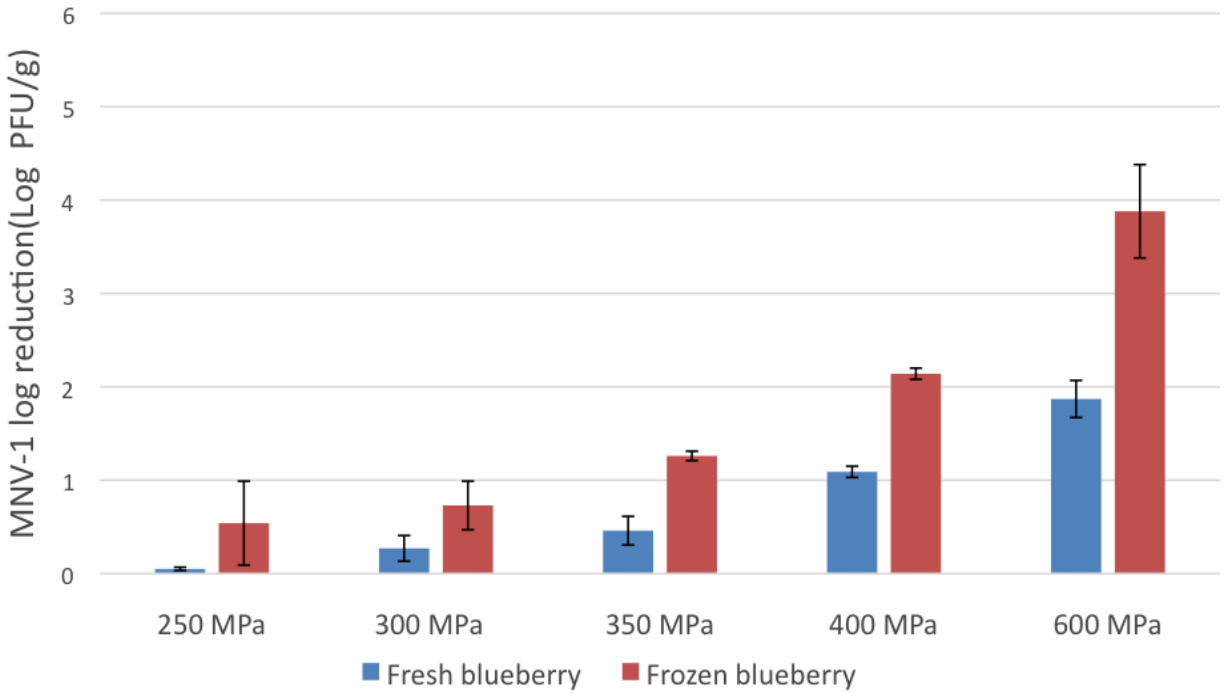
Application of High Pressure Processing (HPP) on Fresh and Frozen Strawberries

	Fresh control	No HPP (pouched)	400 MPa, 1 min	600 MPa, 5 min
Fresh				
Comments	5/5 - Fresh red color, firm berries, bright, healthy cells on internal cut surface.	5/5 - Same appearance as Fresh control berries.	4/5 - Red color of skin is intact after processing. Skin may be slightly darker than No HPP, but no substantial change. Color leaches from flesh, so cut surfaces are duller and less vibrant than No HPP. No difference between pressure treatments.	
	Frozen control	**No HPP (pouched)**	**400 MPa, 1 min**	**600 MPa, 5 min**
Frozen				
Comments	3.5/5 - Relatively firm, but softer than fresh berries.	3.5/5 – Same appearance as Frozen control berries.	2/5 - Berries softer than No HPP. Flesh color slightly duller, skin slightly darker than the no HPP control. No difference between pressure treatments.	

HPP Inactivation of MNV on Fresh and Frozen Strawberries and Blueberries

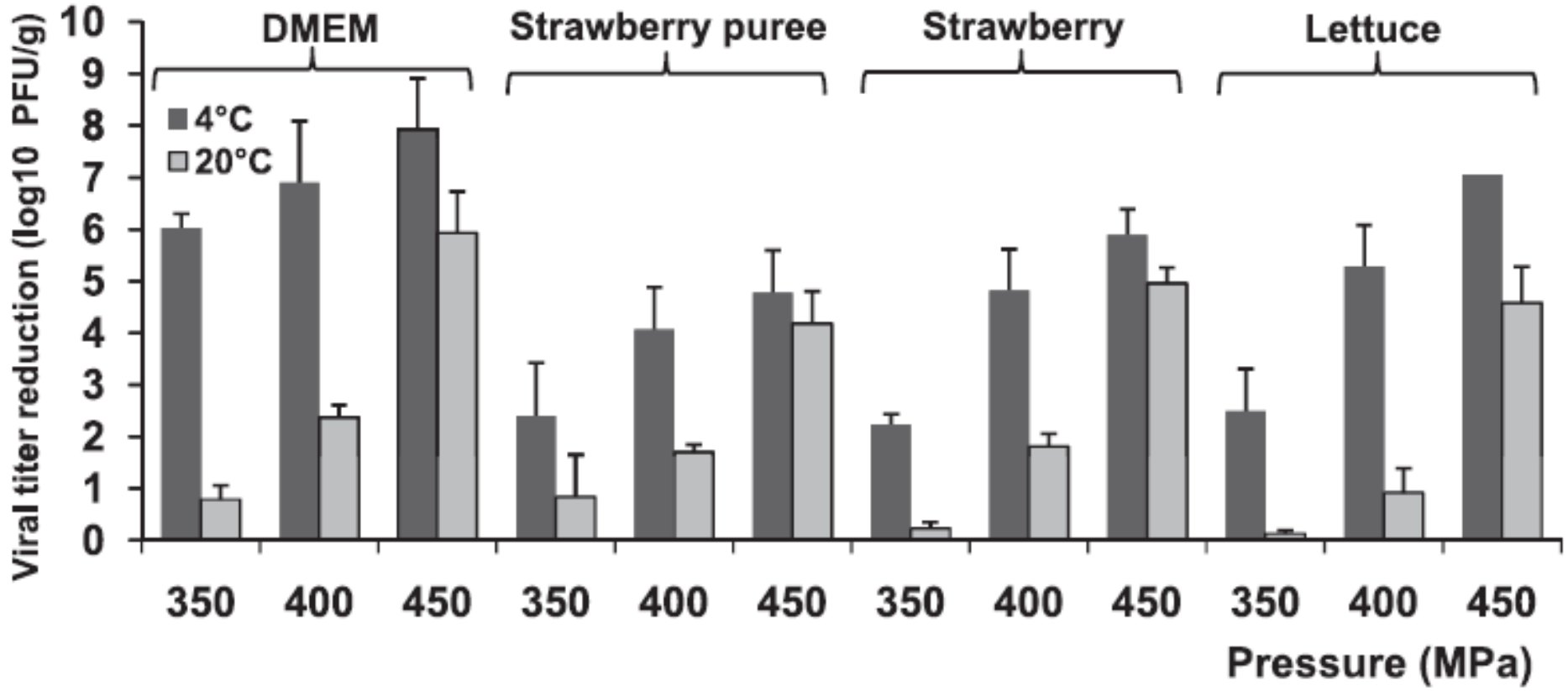


Strawberry



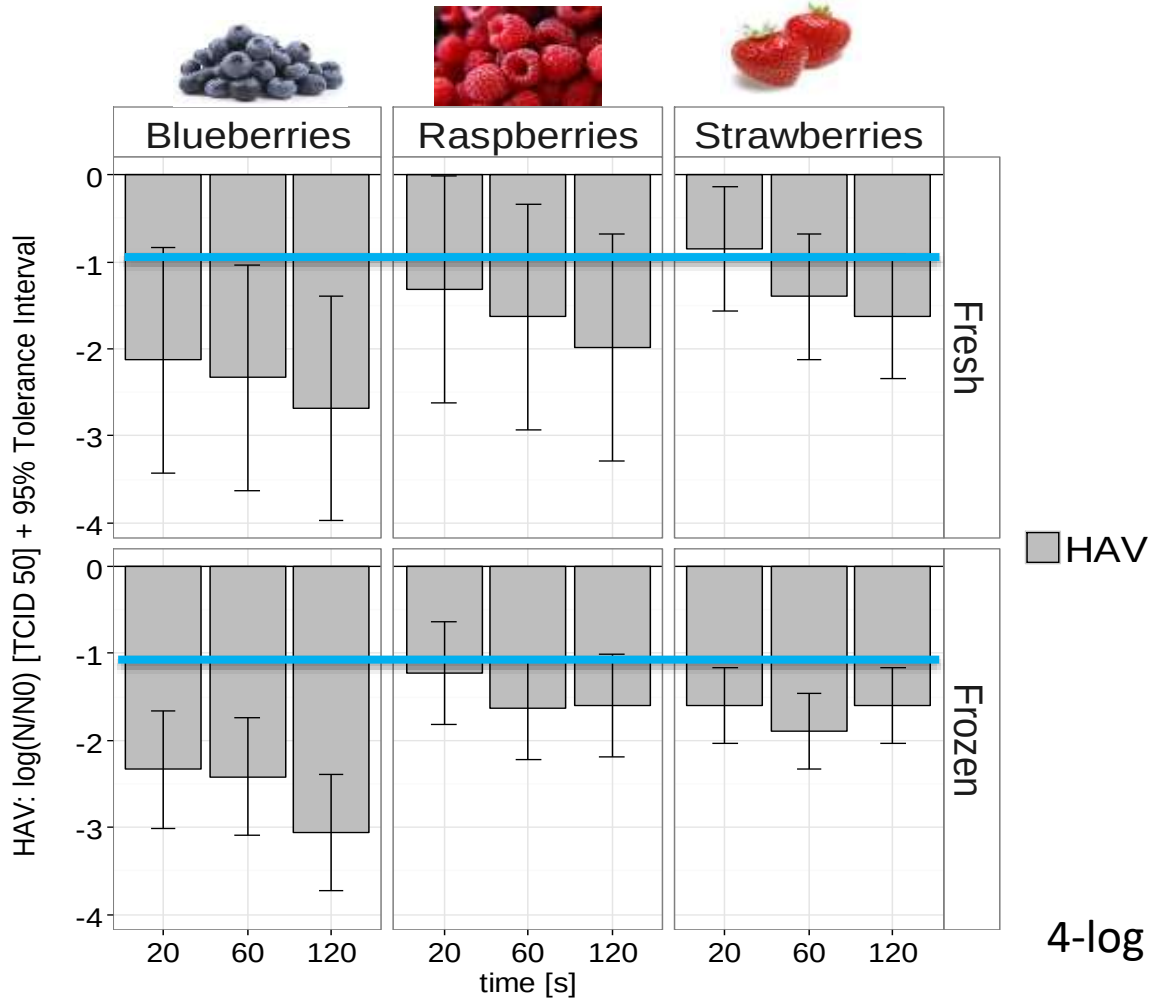
Blueberry

MNV-1 Inactivation by HPP in Various Food Matrices



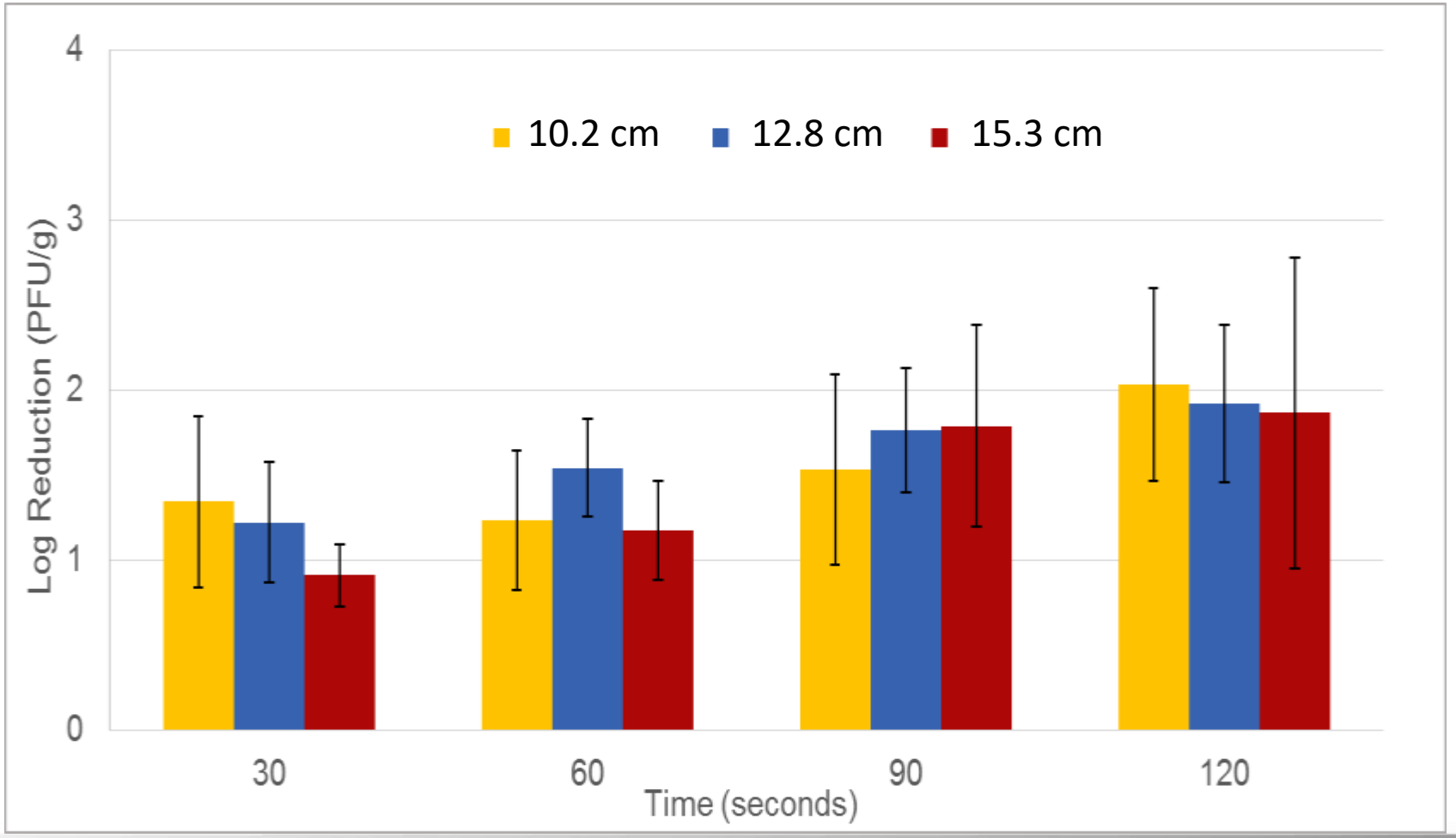
Lou *et al.*, 2011, AEM 77(1862-1871)

Hepatitis A Virus Inactivation by UV-C on Fresh and Frozen Berries



4-log initial

Inactivation of MNV-1 on Fresh Strawberries using Pulsed Light (3 Hz)



4 log inoculation

Highlights of Using Surrogates in Processing Technologies

Processing Technology	Possible Viral Inactivation Mechanism	Inactivation of Surrogates
Frozen and chilled storage	Instability of viral capsid	• Low reduction of most surrogates. • Viruses stable in most frozen or chilled conditions.
pH and water activity	Unknown, if any	• Low reduction of most surrogates, except FCV which is pH sensitive and thus not an appropriate surrogate for acidic matrices.
Light based technologies	Photochemical reactions may cause capsid instability	• High inactivation in clear liquids and on surfaces of most surrogates. • Low inactivation on complex food surfaces or turbid liquids or liquids containing particles. • Low penetration depth and reduced inactivation if viruses are in food matrices.

- Currently used / applied food processing technologies can be classified in achieving either around 1 $\log_{10}$ (“low”) or around 3 $\log_{10}$ (“high”) reductions, however, the choice of surrogate may result in significant differences

Outlook on Hepatitis E Virus

HEV

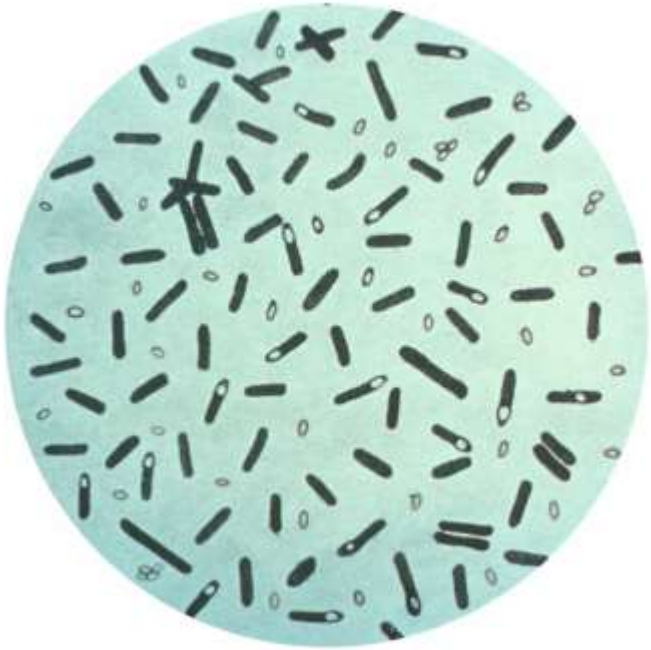
thermal processsing

Control measures	Matrix	Virus	Reduction	Reference
71°C for 20min	Pig liver	HEV	Complete inactivation (pig model)	Barnaud *et al*., 2012
62°C for 30 min **72°C for 30 sec** **100°C for 3 min**	Cow milk	HEV	Incomplete inactivation (monkey model) Incomplete inactivation (monkey model) Complete inactivation (monkey model)	Huang *et al.*, 2016
70°C for 1.5 min	Cell culture medium	HEV	3.6 $\log_{10}$	Johne *et al*., 2016

- Inactivation data needed of HEV in meat and milk at the different time-temperature combinations used during processing

Clostridium botulinum

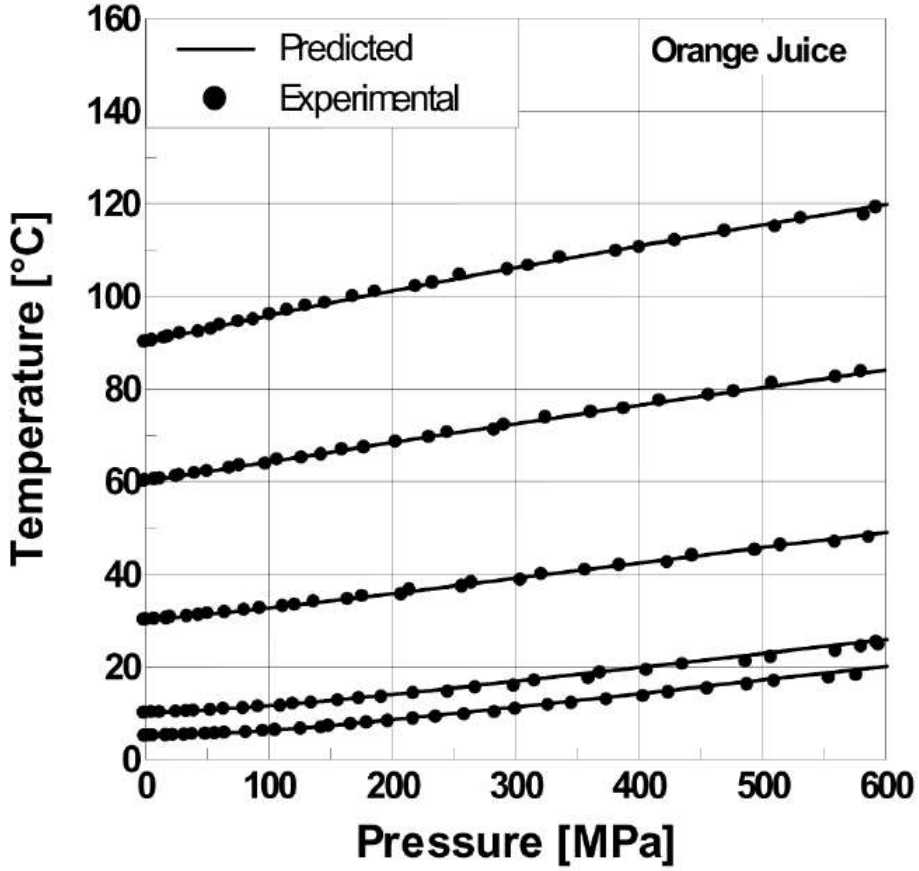
- Anaerobic, Gram positive, rod-shaped spore-forming bacteria
- Growth pH 4.8-7.0
- Lipase negative, proteolytic/nonproteolytic
- Spores resistant to heat and UV light and can remain dormant for years
- Produces botulinum toxin A-G, a 150kDa two-stranded protein of extremely potent poison to humans/animals



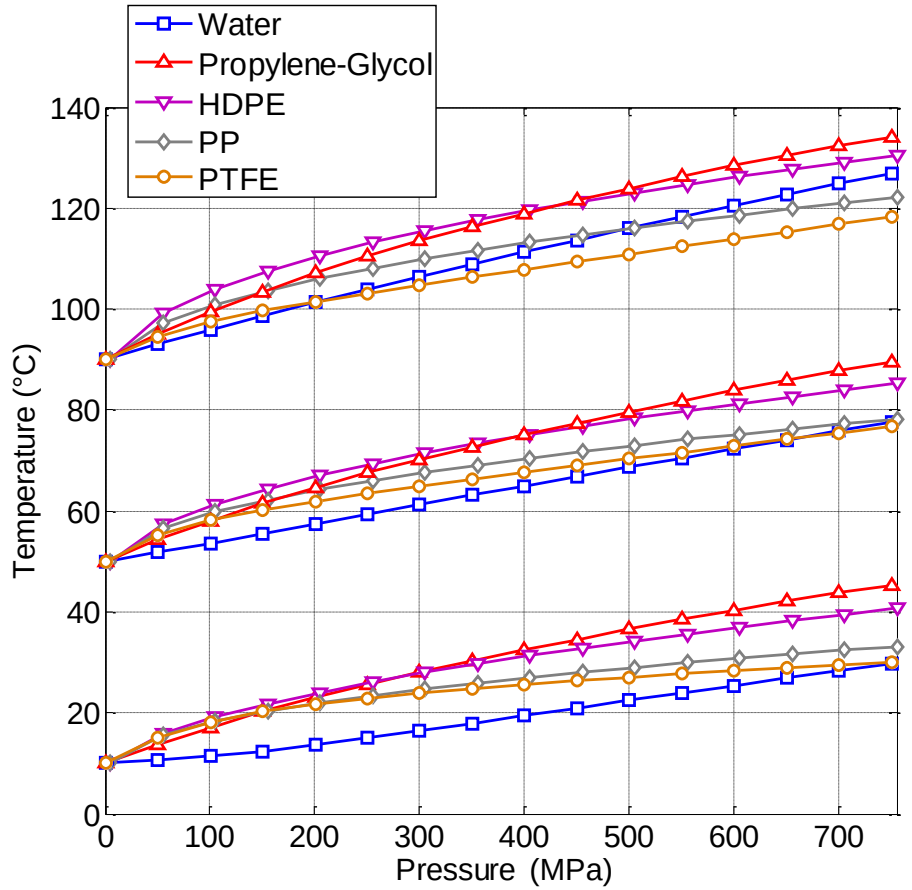
C. botulinum Groups

Group	I	II	III	IV*
Toxin type	A, B, F	B, E, F	C, D	G
Proteolysis	yes	no	no-weak	no
Growth temp Optimum °C	35-40	18-25	40	37
Growth temp Minimum °C	12	3	15	
Disease host	human	human	animal	

Temperature Elevation Due to Pressurization



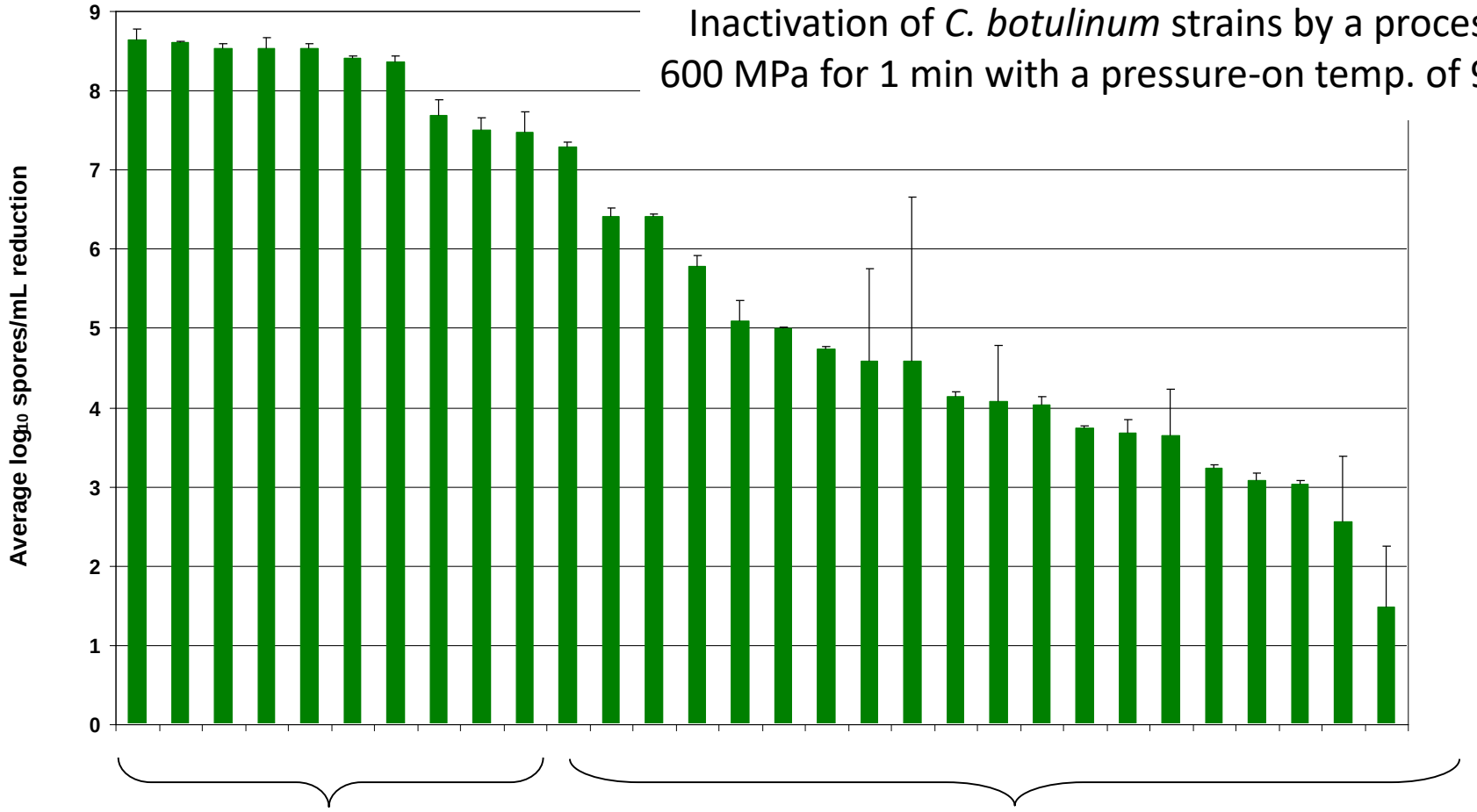
Ardia et al., 2004



Knoerzer and Versteeg, 2009

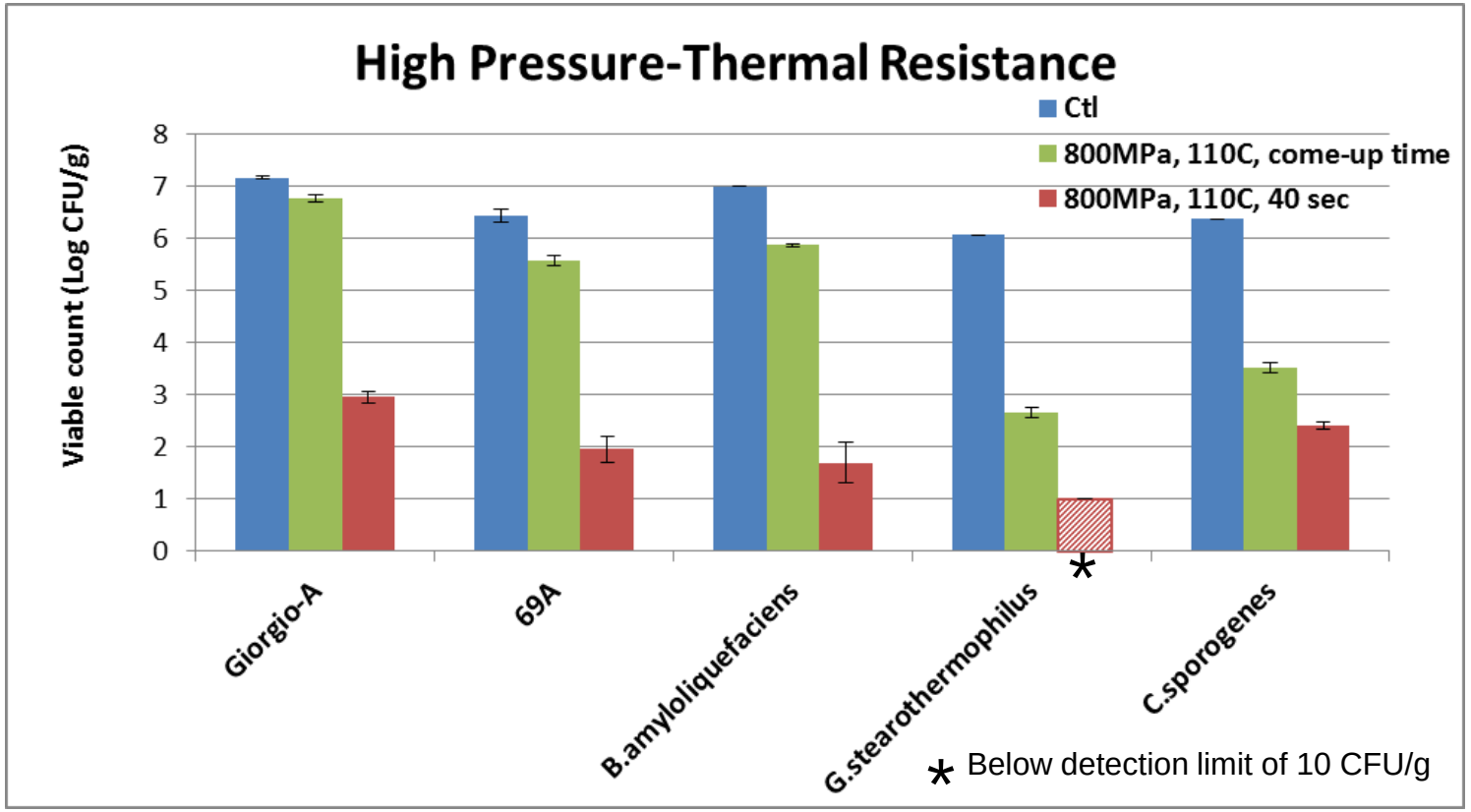
Clostridium botulinum inactivation by HPP

Inactivation of *C. botulinum* strains by a process of 600 MPa for 1 min with a pressure-on temp. of 95° C



Chapman 2007-2008

Characterization and Qualification of Challenge Microorganisms



Comparison of pressure-thermal resistance of spores crops

Challenges for Validation

HuNoV cultured! HEV culturable strain available

Standardized method for evaluating decontamination strategies for foods

A forum for standards development? Who should initiate this development?

ALSO: Surrogates for validations at pilot-scale are lacking

BECAUSE: Guidelines lacking on surrogate choice, inoculum level and inoculation methods

BUT: Cultivable human NoV and HEV not widely available yet and quantification of inactivation levels above 3 log_{10} may be difficult to evaluate

Thank You!