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Risk Mitigation in Reducing Thermal Processing with Hurdle Technologies: A Challenge Study in the Canning Industry with *Clostridium botulinum*

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125 ANS de RECHERCHE en AGRICULTURE
YEARS of AGRICULTURAL RESEARCH

S'enraciner dans la science • Innover pour l'avenir
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Canada

Context

- 200th anniversary of Nicolas Apert's book publication
- Fruits and vegetables intake in Canada : still inferior to recommendations

Quality
- Nutritive value
- Texture
- Color
- Taste

\+

« Healthy »

Easy to use

Fresh vegetables	Canned vegetables
✓Nutritive value ✓Texture, Color, Taste -Stock shortage risk -Microbial contamination -Time-consuming	✓Safe ✓Easy to use -Nutritive value -Texture, Color, Taste

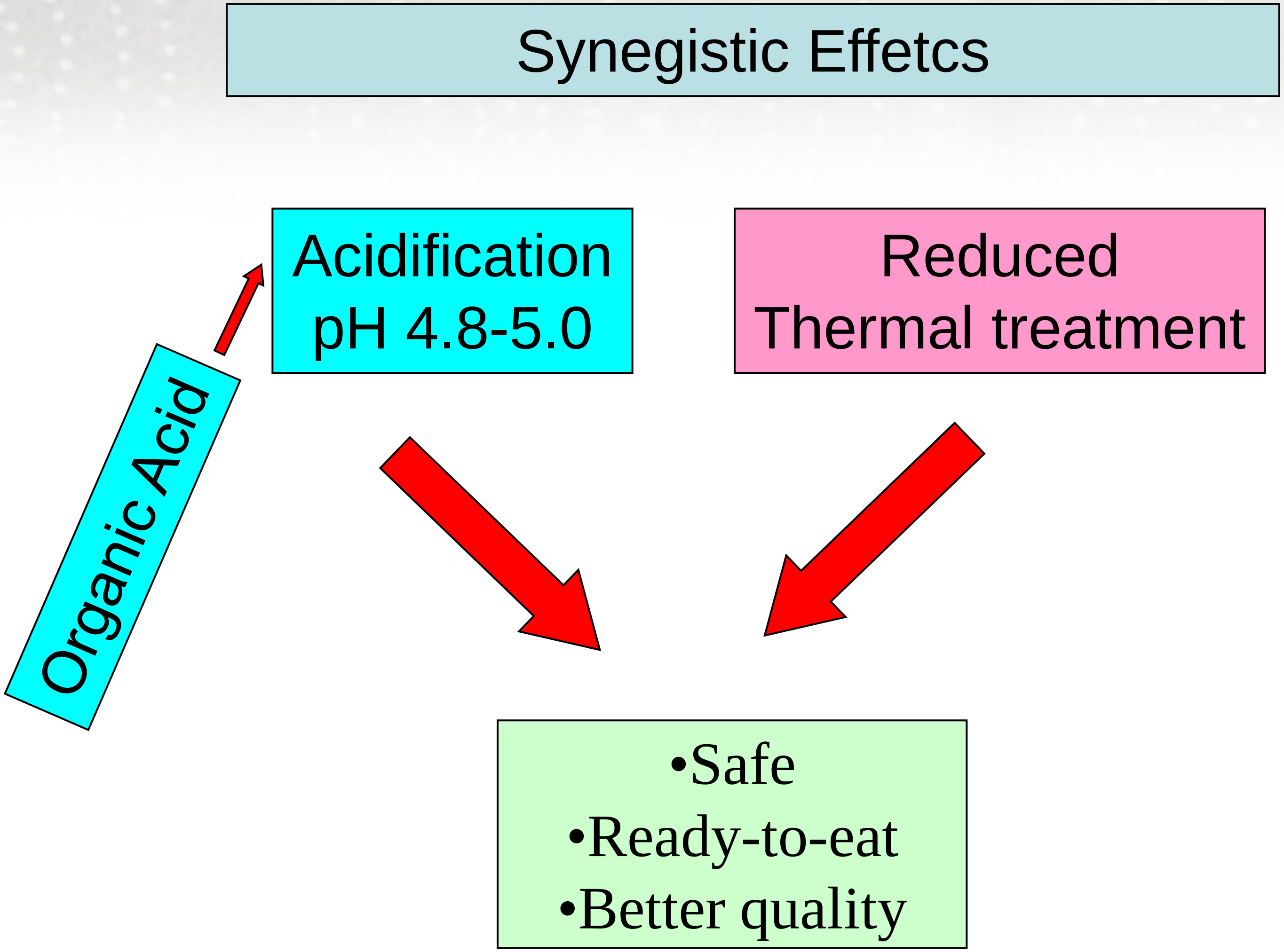
Introduction

- This project has been selected by the Quebec Fruit and Vegetable round table: a committee of federal, provincial governments researchers and vegetables processors suggested innovative ways to improve vegetables offer on the market.

- Canned vegetables are often perceived as overcooked products, mushy or soft, with less nutritional value. We reach the conclusion that a slight acidification combined with reduced thermal process could be a good way to create new markets for canned vegetables as repeatedly suggested many times in literature.

- New theories on thermal processing models and microbial inactivation of spores also appears to justify some research evaluation to meet legislation standards.

Hurdle Technologies



Acidification Advantages

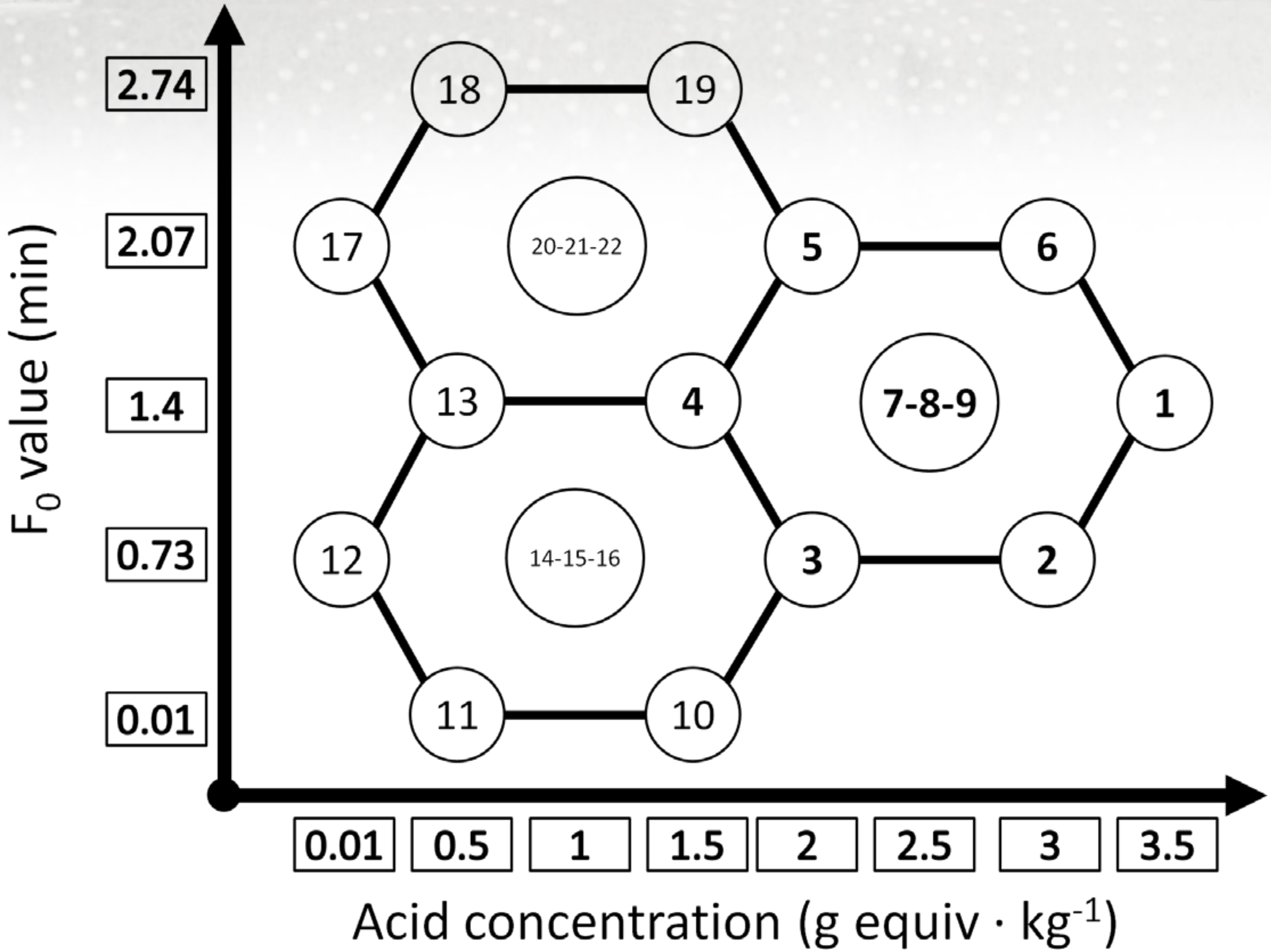
- Effect of the acidification:
  - Thermal resistance of bacteria and bacterial spores (D--value): decrease with the pH
  - Acidification inhibit recovery and germination of injured spores
- D-z values in the literature are mainly measured:
  - At pH 7 (maximum resistance)
  - In water or other liquid media (phosphate buffer, tryptone)
- A few data are available on bacterial inactivation kinetics:
  - In the pH 4.6-5.0 range
  - In real food matrix

Aim of the Project

This project was mostly directed to:

1. Review of the calculation models used to define the sterilization processes for canned vegetables.
2. Evaluate the thermoresistance of pathogen and spoilage microorganisms, and compare the destruction kinetics models.
3. Determine the technical feasibility of minimally processed vegetables in cans (pH 4.8 and 5.0, using different acidulant types and concentrations with minimal thermal treatment), including sensory evaluation tests.
4. Microbiologically validate the efficiency of processes combining minimal thermal treatments and acidification at the pilot-scale study.

Shell Experimental Design



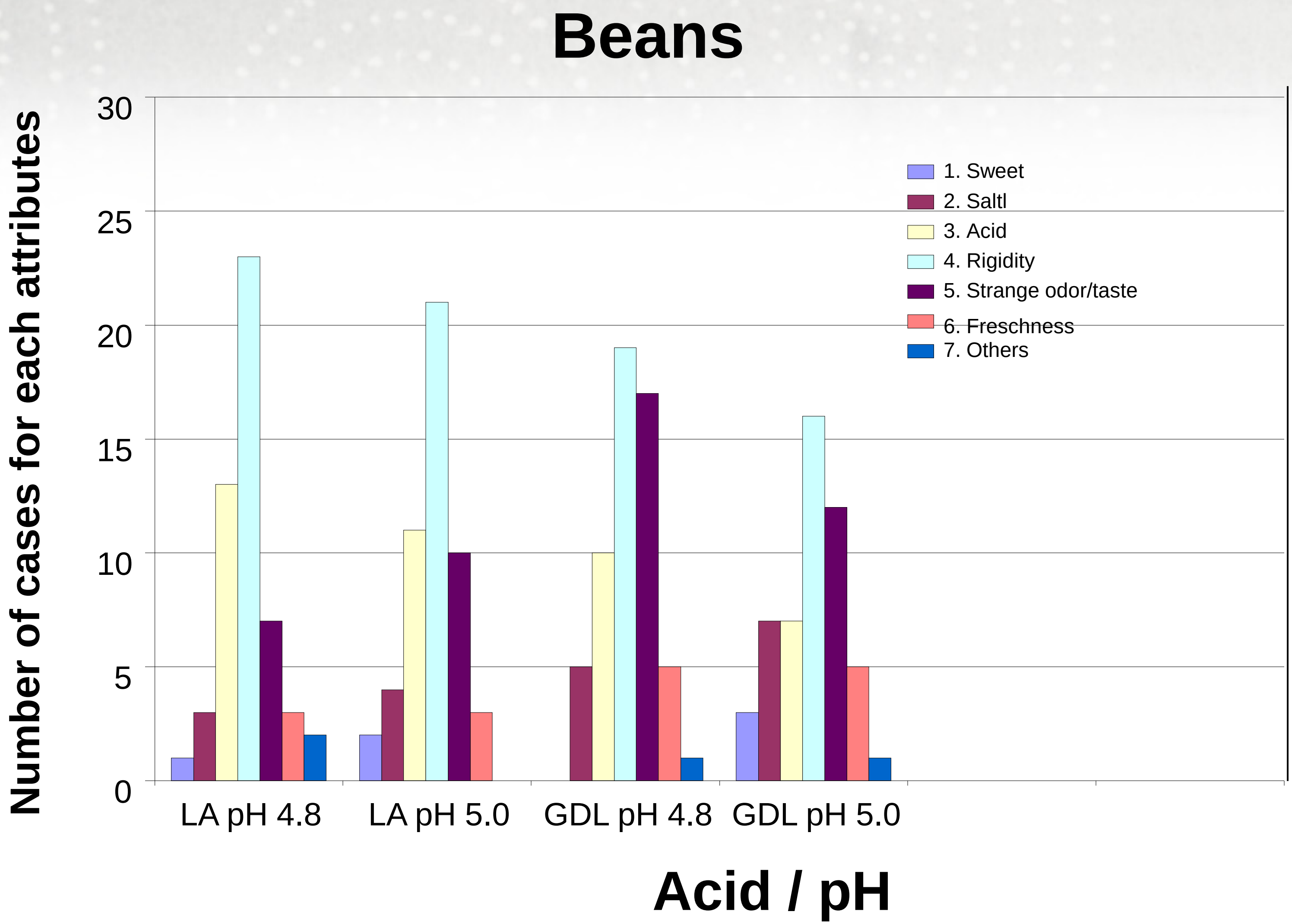
Shell Experimental Design

Example of uniform shell experimental design with two independent variables: target lethality (F_o) and acid concentration (C). It is a 2 factorial experiment with 5 levels of C (1 central, 2 higher and 2 lower levels); and 3 levels of F_o (1 central, 1 lower and 1 higher level).

	First Set			Second Set			Third Set		
Coded	No of Exp	C(%)	F_o	No of Exp	C (%)	F_o	No of Exp	C (%)	F_o
-2	4	1,5	1,4	12	0,01	0,73	17	0,01	2,07
-1	3	2	0,73	13	0,5	1,4	18	0,5	2,74
-1	5	2	2,07	11	0,5	0,01	13	0,5	1,4
0	7	2,5	1,4	14	1	0,73	20	1	2,07
0	8	2,5	1,4	15	1	0,73	21	1	2,07
0	9	2,5	1,4	16	1	0,73	22	1	2,07
1	6	3	2,07	4	1,5	1,4	4	1,5	1,4
1	2	3	0,73	10	1,5	0,01	19	1,5	2,74
2	1	3,5	1,4	3	2	0,73	5	2	2,07

The levels of each variable could be unlimited, replicated experiments are in red.

Sensory Evaluation for Lactic Acid and GDL



Microbial Kinetics Design

Spores

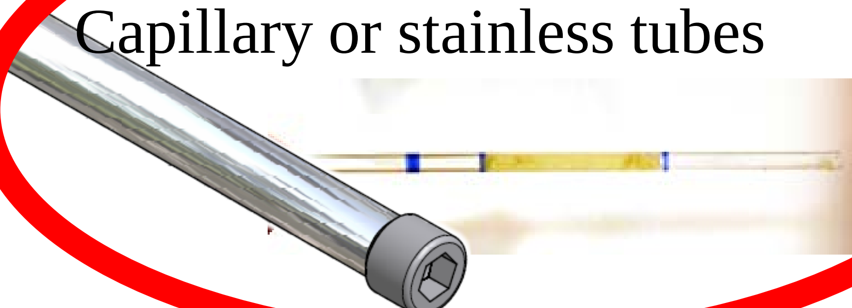
Food matrix
(green beans puree, brine)

Acidulant
(LA, GDL c.a. pH 4.8)

D and z values determination

Inoculation

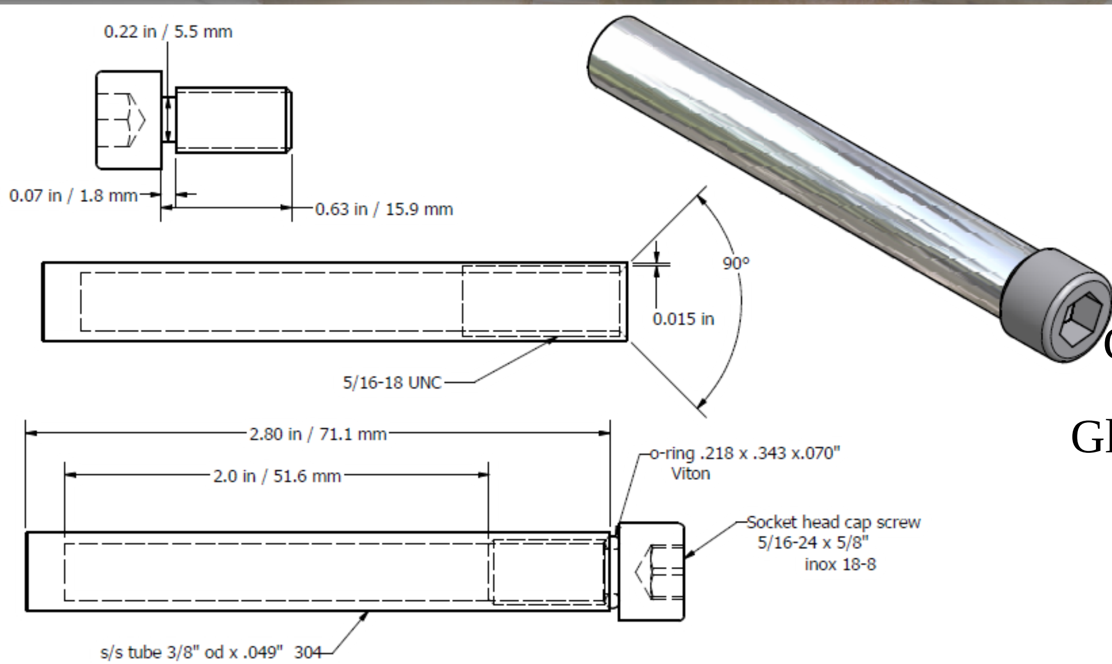
Capillary or stainless tubes

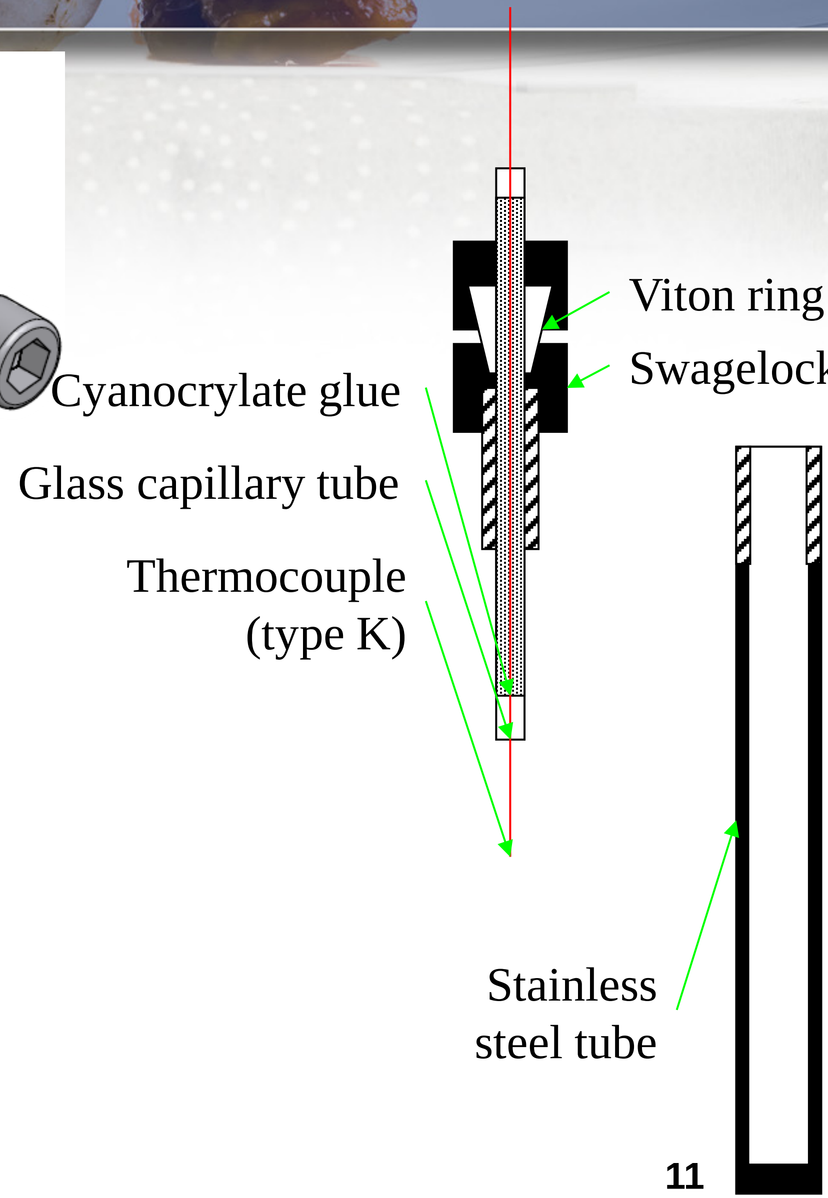


Count surviving spores

Treatment

Stainless Tubes for BioSafety Consideration





Microbiological Methods

- 3 Food matrices Green beans in brine non-acidified (pH ≈ 5.8), acidified with Lactic acid or with GDL (pH ≈ 4.8)

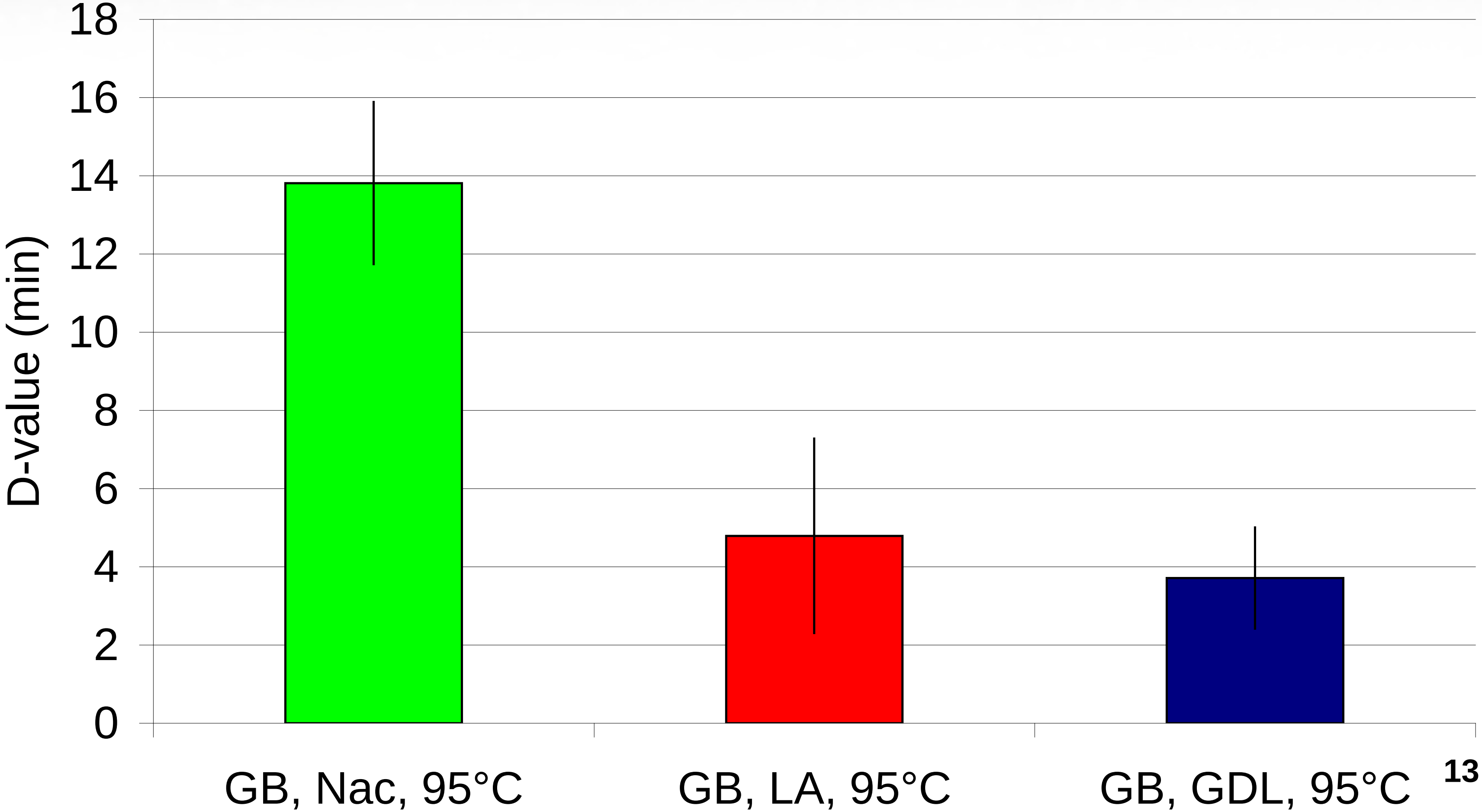
- 3 Groups of microorganisms

- 2 Levels of experimentation in Tubes (Glass/Stainless) and in Cans

	***Clostridium sporogenes* (surrogate)**	***Clostridium botulinum* *(cocktail)***	***Geobacillus stearothermophilus* (spoilage)**
Strain	PA 3679 (ATCC #7955)	62-A, PC0101AJ0, 13983B	ATCC #12980
Spore production	RCM, 37° C, anaerobiosis	RCM, 37° C, anaerobiosis	SM, 60° C, aerobiosis
Enumeration	RCM	RCM	TSA

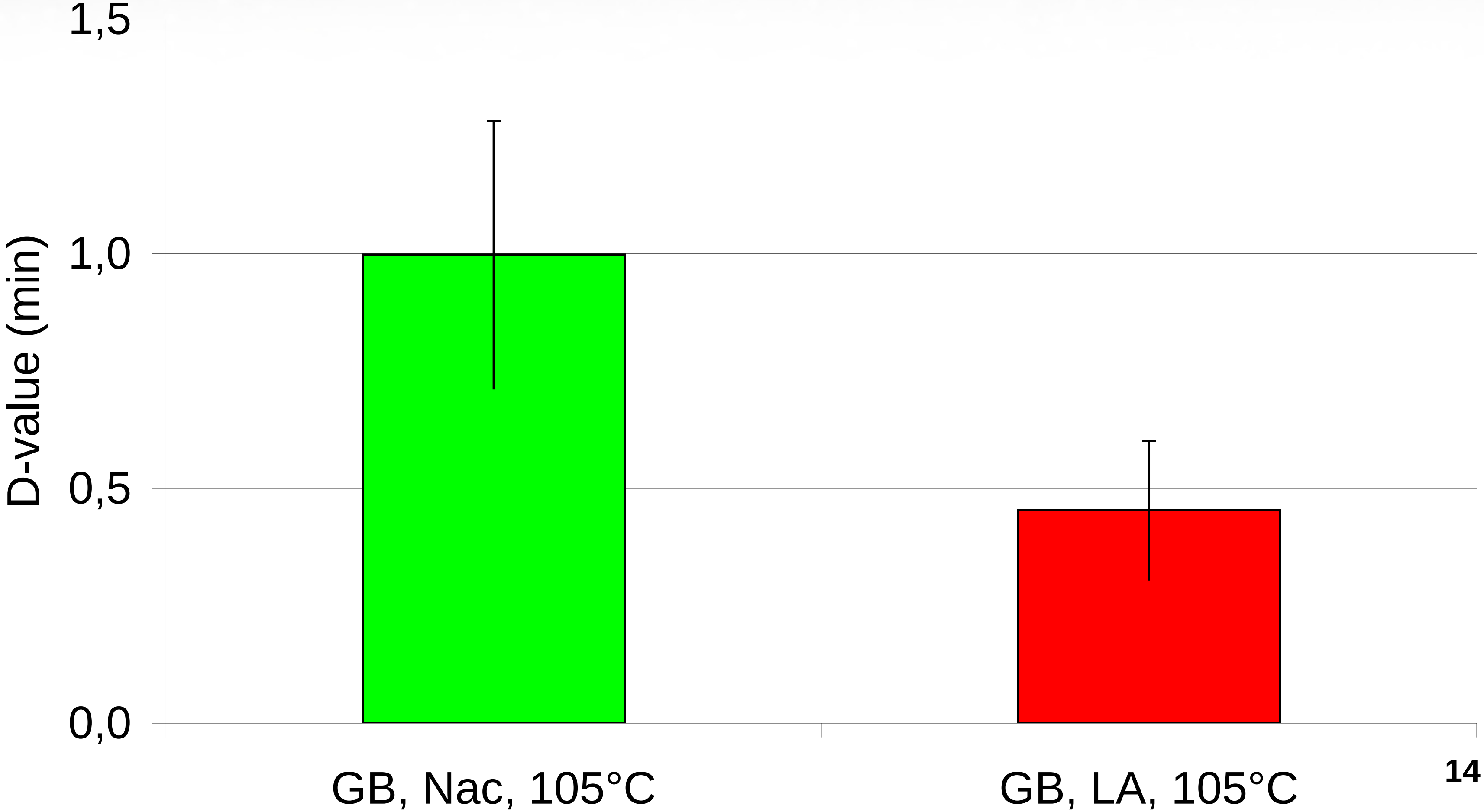
Results for *Clostridium Sporogenes* PA3679 (Tubes)

D-values at 95°C of *C. sporogenes* PA 3679 in Green Beans, Non acidified and acidified with LA c.a. pH 5.0



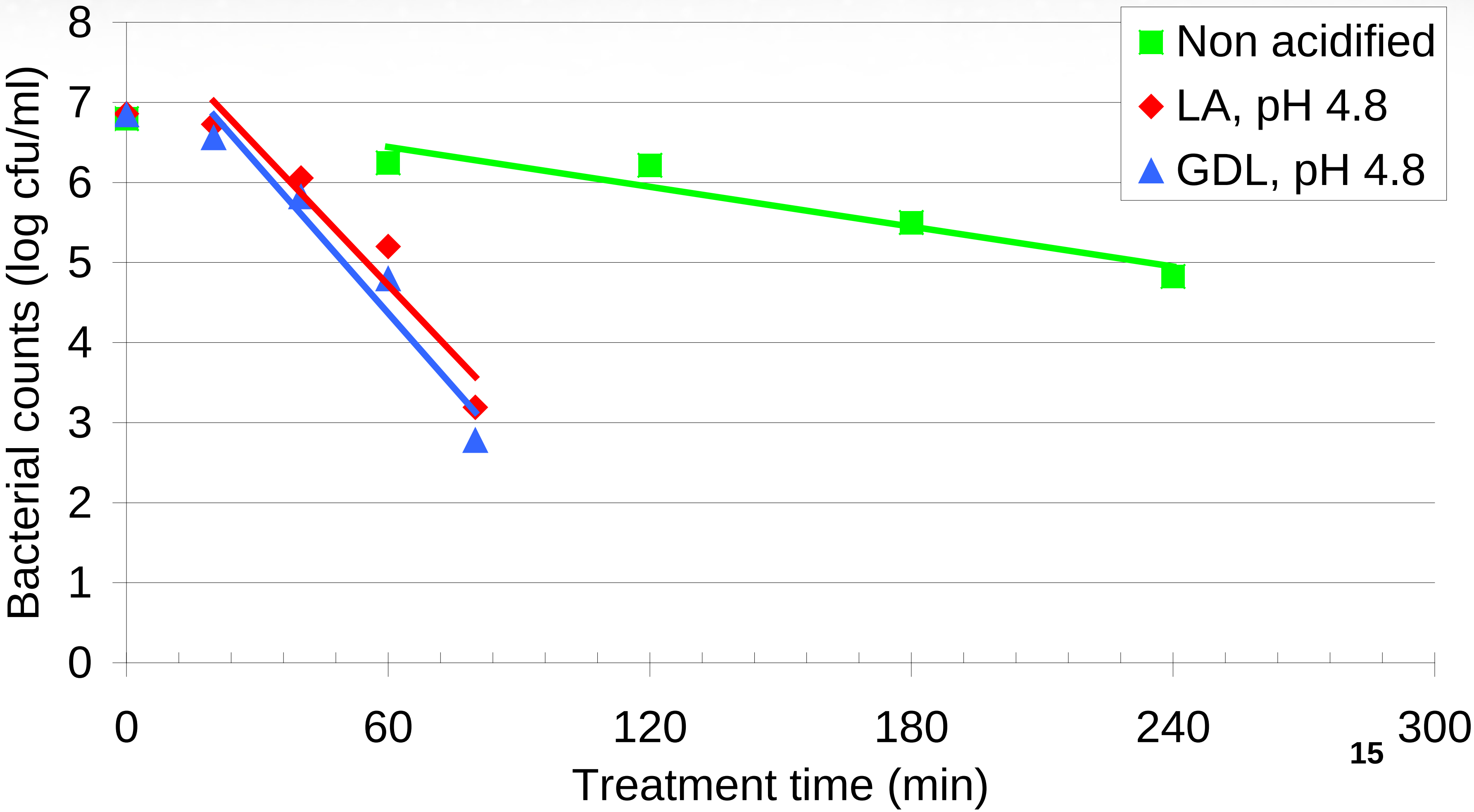
Results Clostridium Sporogenes PA3679 (Tubes)

D-values at 105°C of *C. sporogenes* PA 3679 in Green Beans, Non acidified and acidified with LA c.a. pH 5.0



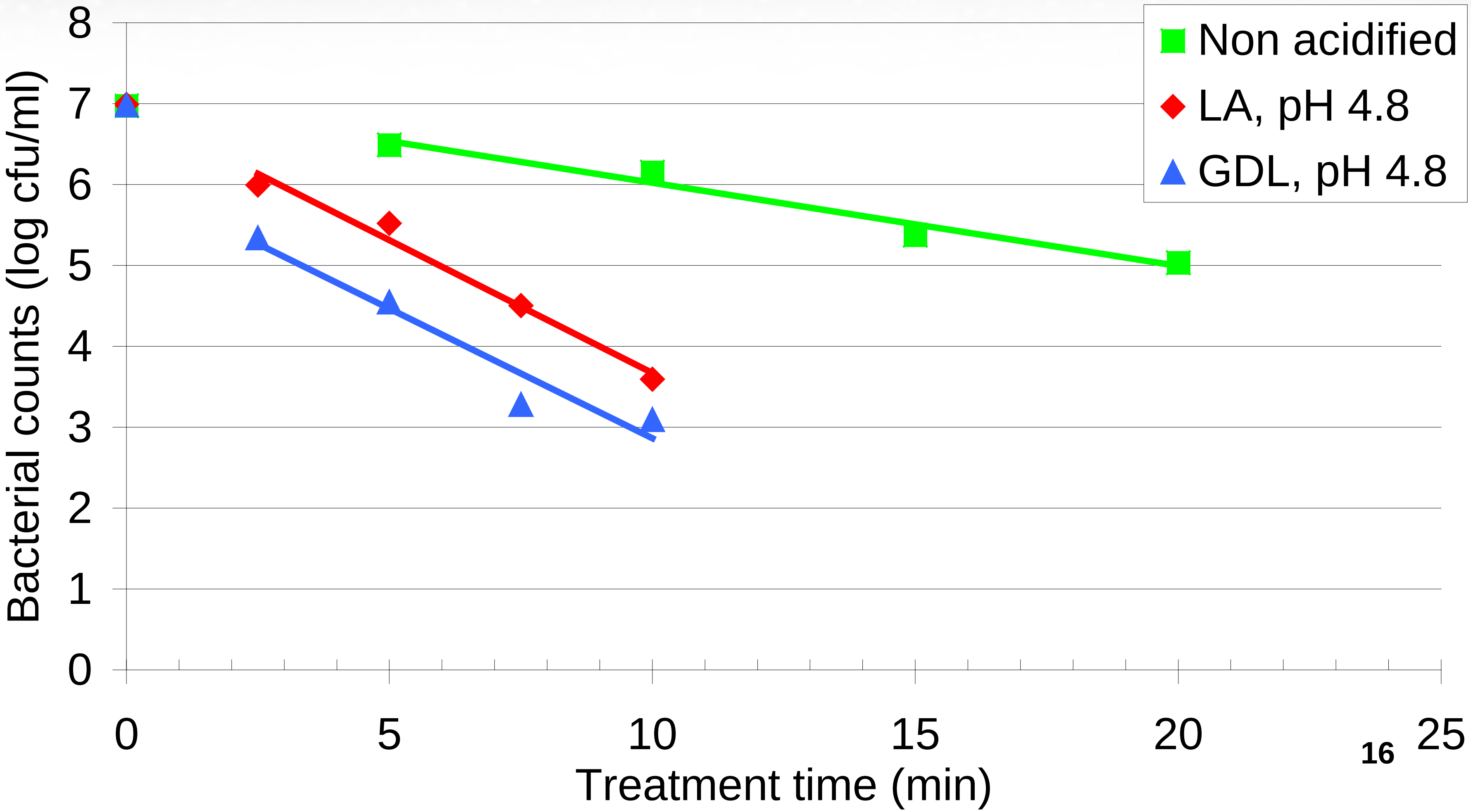
Results for *Clostridium botulinum* Cocktail (Tubes)

Inactivation kinetics of *C. botulinum* spores mix in green beans puree at 95°C



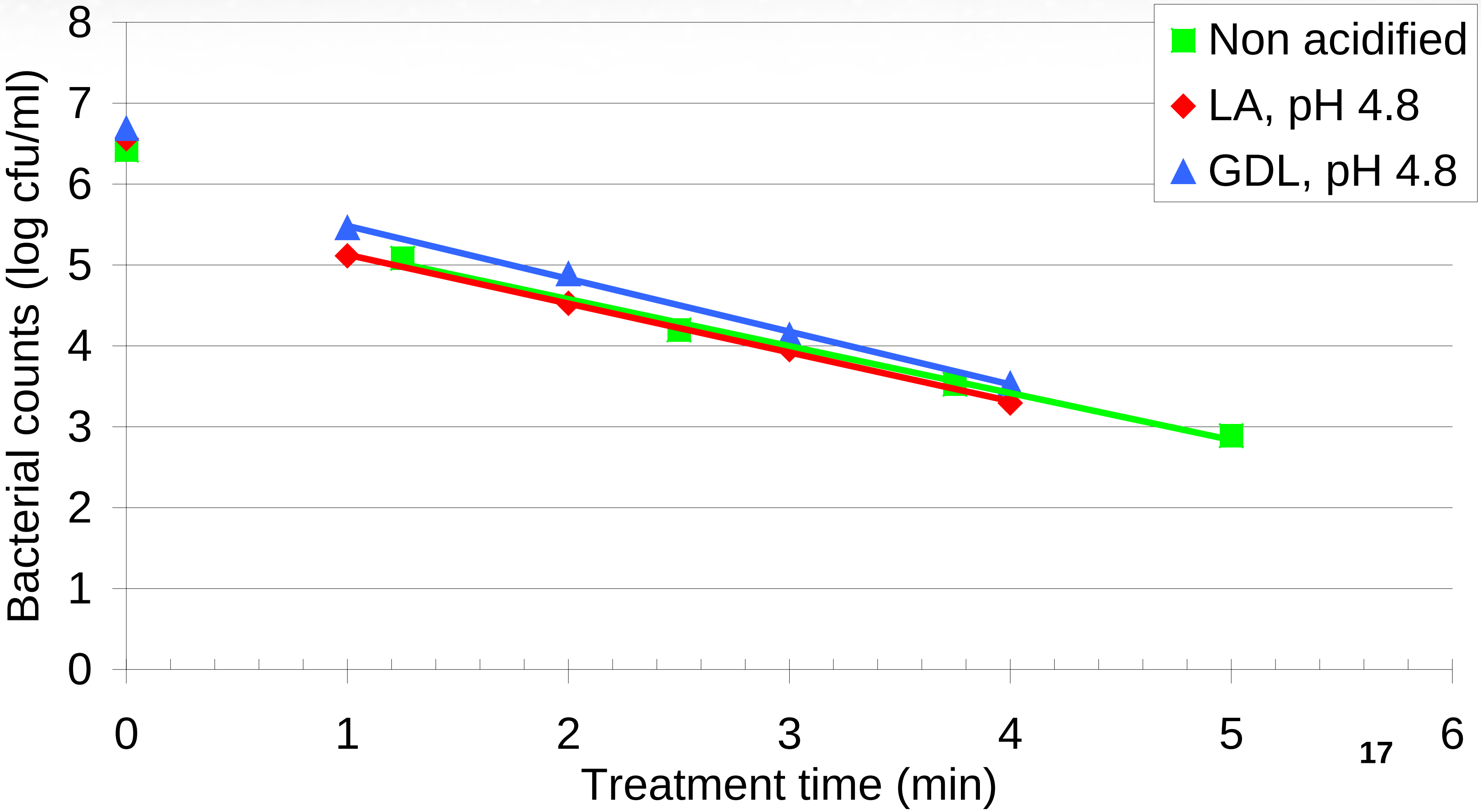
Results for *Clostridium botulinum* Cocktail (Tubes)

Inactivation kinetics of *C. botulinum* spores mix in green beans puree at 100°C

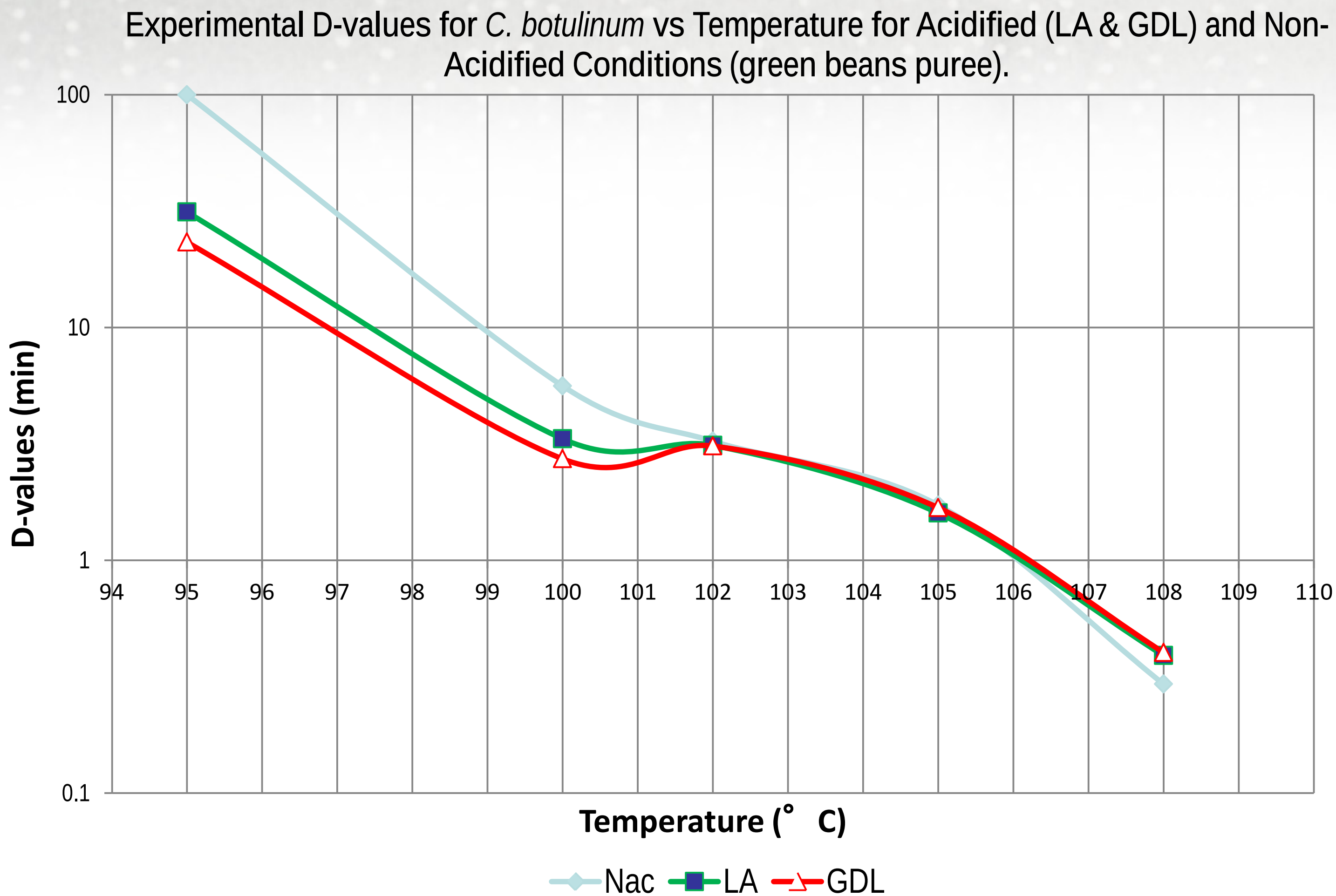


Results for *Clostridium botulinum* Cocktail (Tubes)

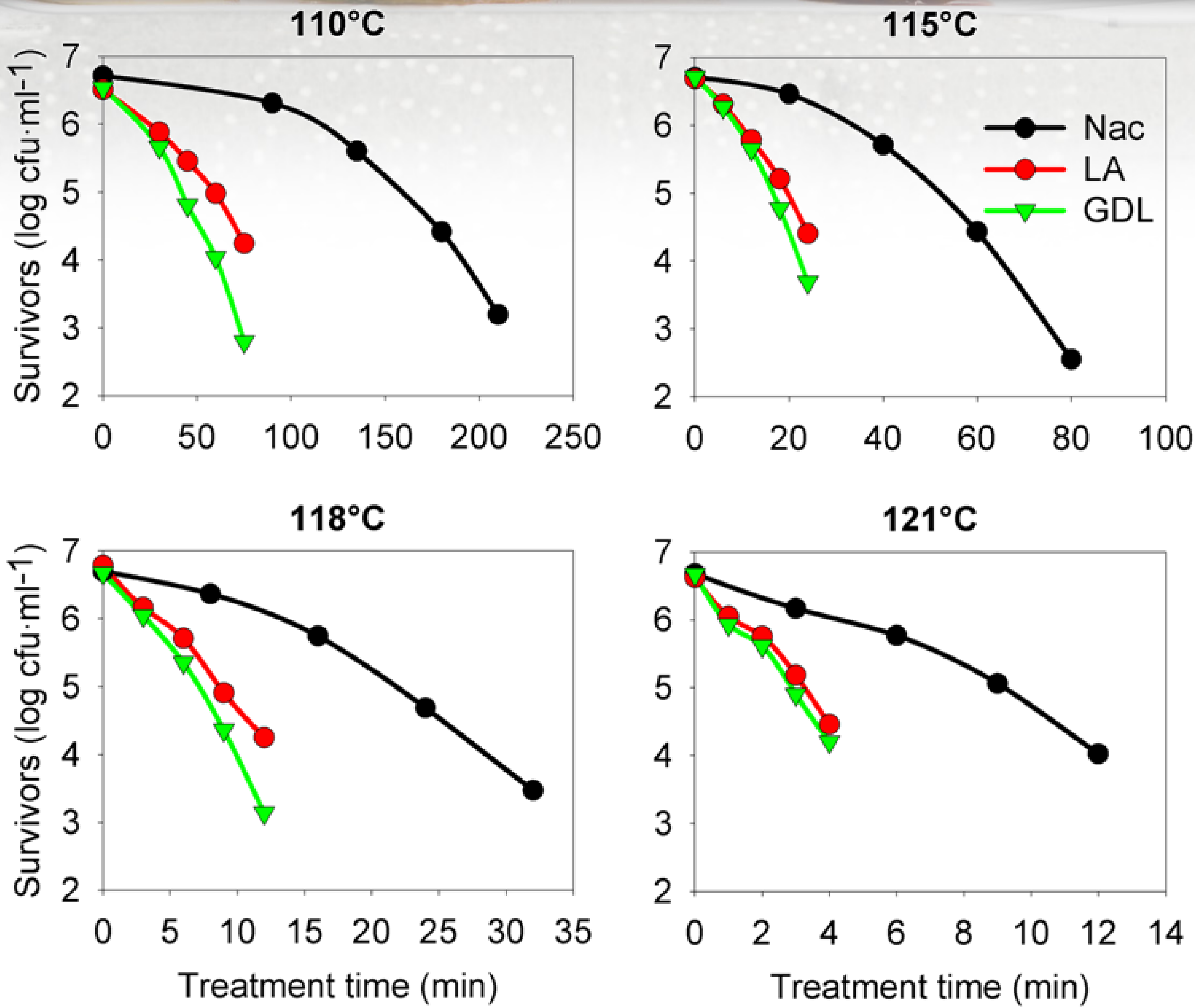
Inactivation kinetics of *C. botulinum* spores mix in green beans puree at 105°C



Results for *Clostridium botulinum* Cocktail (Tubes)



Results for *Geobacillus stearothermophilus* (Tubes) #12980



Recorded D-Values

Clostridium sporogenes

Température (°C)	Fèves				
	Nac	LA		GDL	
		4.8	5.0	4.8	5.0
95	13.81 ± 2.07 ^{a}	4.47 ± 0.42 ^{b}	4.79 ± 2.49 ^{b}	4.03 ± 0.47 ^{b}	3.71 ± 1.3 ^{b}
105	1 ± 0.28 ^{a}	-	0.45 ± 0.15 ^{a}	-	-

Clostridium botulinum cocktail

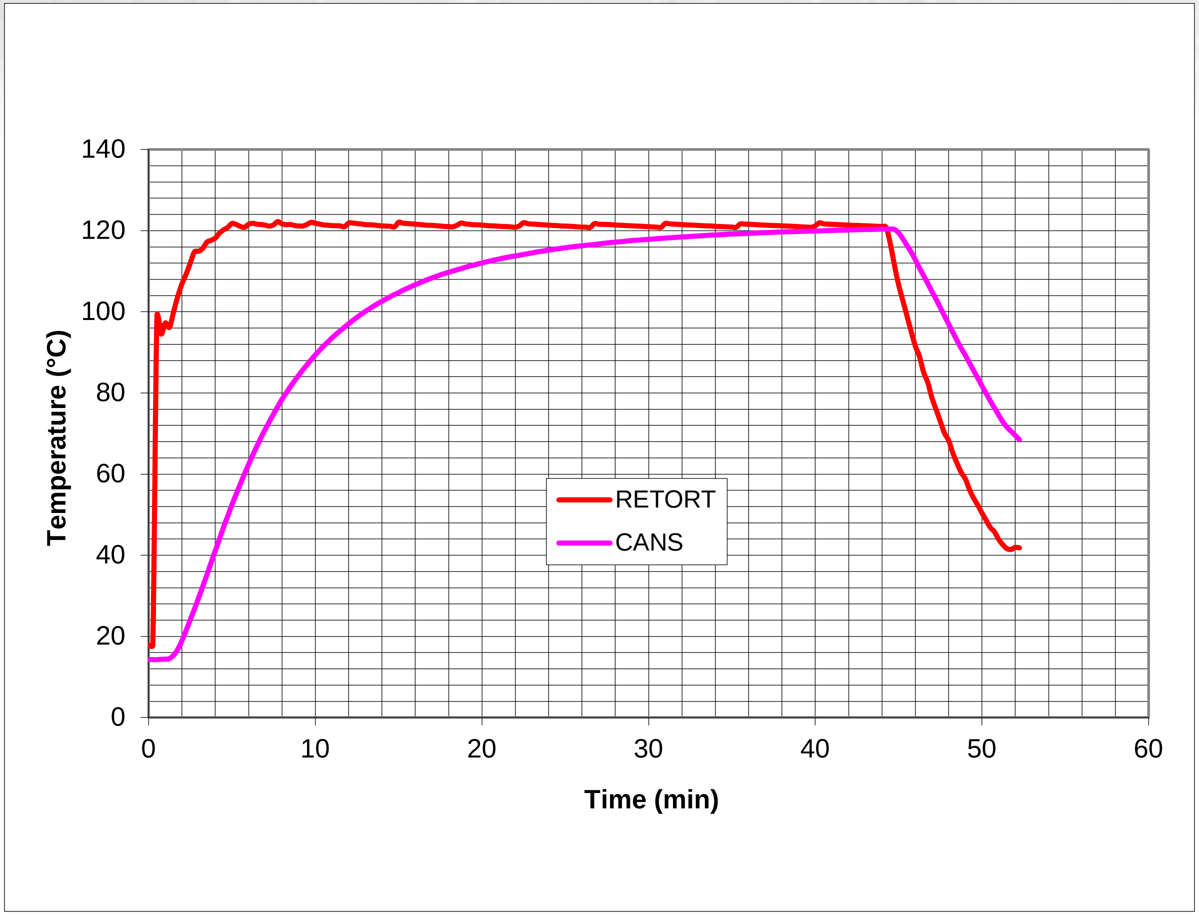
	Valeurs D moyennes (min) ± écart-type		
Température (°C)	**Nac**	**LA**	**GDL**
95	99.87 ± 18.06 ^{a}	31.29 ± 2.79 ^{b}	23.24 ± 3.19 ^{b}
100	5.61 ± 0.37 ^{a}	3.32 ± 0.30 ^{b}	2.73 ± 0.37 ^{b}
102	3.25 ± 0.15 ^{a}	3.12 ± 0.37 ^{a}	3.10 ± 0.85 ^{a}
105	1.72 ± 0.12 ^{a}	1.6 ± 0.19 ^{a}	1.68 ± 0.29 ^{a}

Geobacillus stearothermophilus

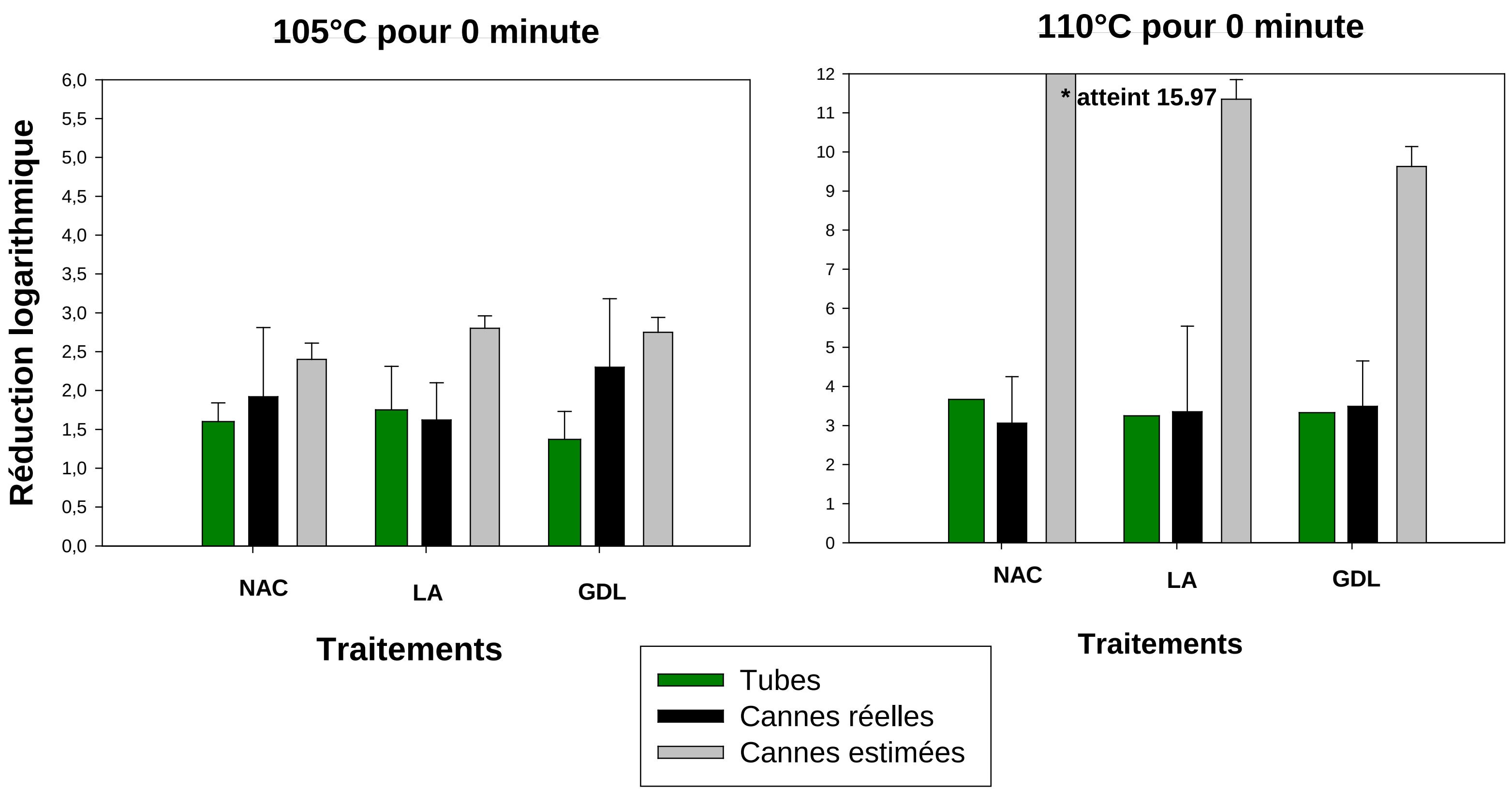
		Valeur D (min) ± écart-type		
	Condition	**NAC**	**GDL**	**LA**
Température (°C)	110	39,66 ± 1,77 ^{a}	21,49 ± 0,89 ^{b}	29,64 ± 2,06 ^{c}
	115	14,82 ± 0,18 ^{a}	6,82 ± 0,14 ^{b}	8,98 ± 0,47 ^{c}
	118	8,10 ± 0,11 ^{a}	3,20 ± 0,15 ^{b}	4,13 ± 0,37 ^{c}
	121	4,01 ± 0,05 ^{a}	1,64 ± 0,16 ^{b}	1,82 ± 0,05 ^{b}
	123	2,49 ± 0,26 ^{a}	0,97 ± 0,12 ^{b}	1,10 ± 0,11 ^{b}
	z (°C)	10,81	9,70	9,03

Bio-Validation in Cans (14 oz)

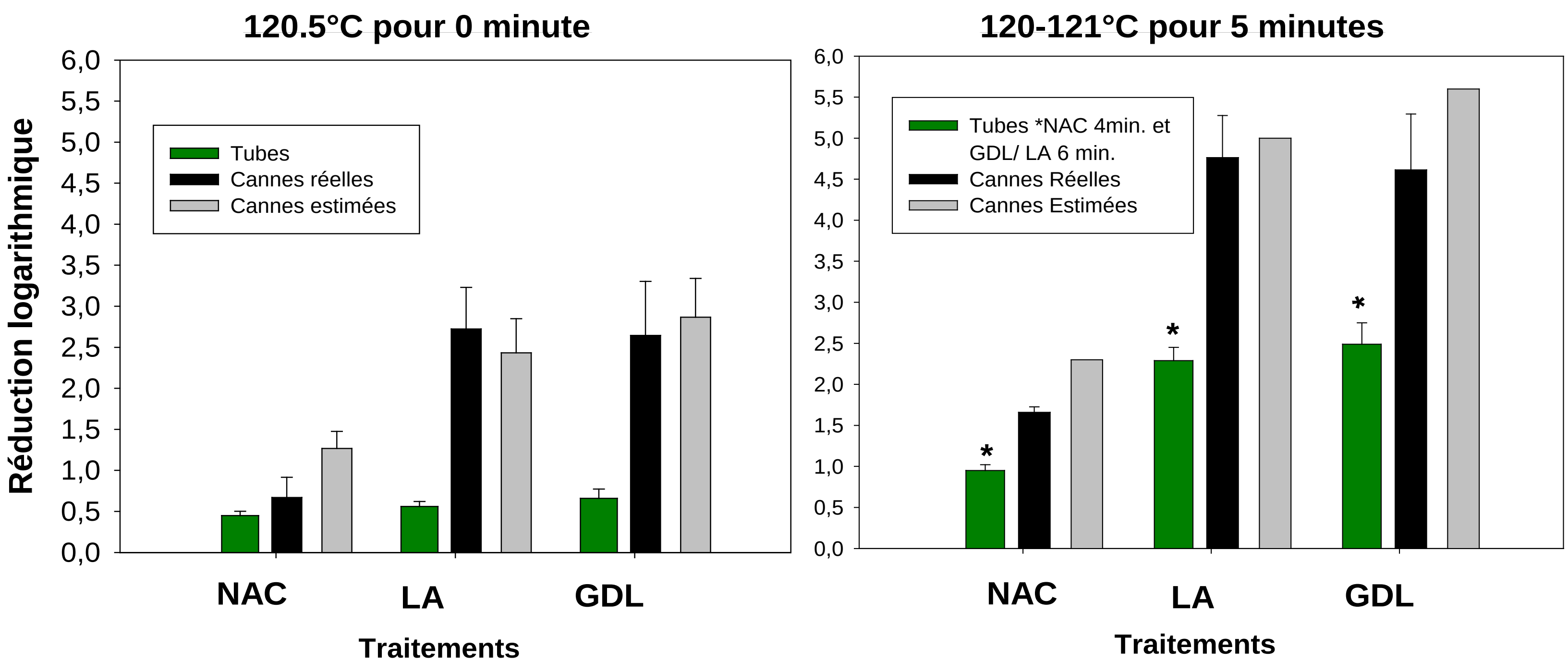
Temperatures Record



Results for *Clostridium botulinum* Cocktail (Cans)



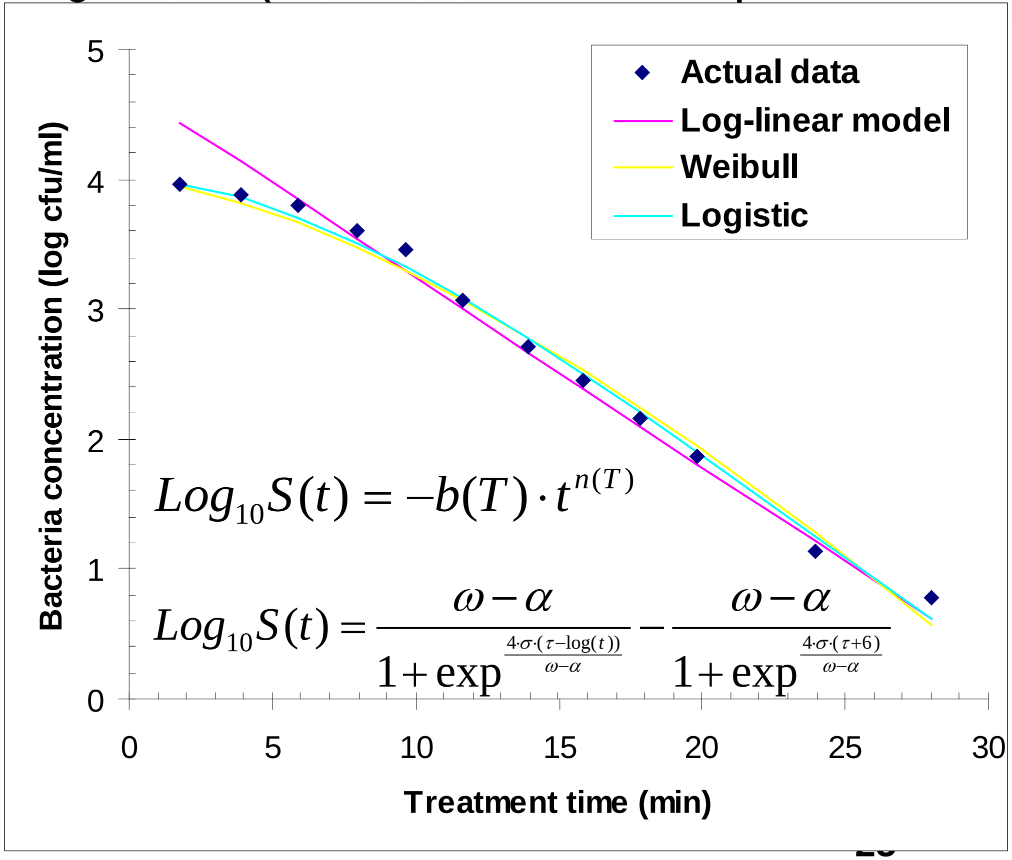
Results for *Geobacillus stearothermophilus* (Cans) #12980



Destruction Kinetics Models

- Limits of the log-linear model
  - Ease of use, no need of complex computations
  - Many survival curves are not log-linear (concave, convex, biphasic, sigmoid, shoulders, tailing)

- Other models were developed
  - Biphasic
  - Multiexponential decay
  - Logistic
  - Weibull
  - Neural networks
  - …



(Lopez et al., 1996, *G. stearothermophilus* spores in water, 120˚ C)

D-values of *C. botulinum*: Comparison of Experimental Data with Existing Models

C. botulinum	Model: Stumbo, 1973	**Experiments**	Model: Mafart and Leguerinel, 1998	Model : Gaillard et al., 1998
Temp., degC	D(pH=7), min	LA (pH=4.8), min	D(pH=4.8), min	D(pH=4.8), min
121.1	0.21	0.11	0.089	0.0504
110	2.71	1.5	1.14	0.6504
105	8.6	3.4	3.6	2.064
100	27.1	9.9	11.4	6.504
95	85.5	33	35.8	20.52

Our experimental data show:

1) Under acidified conditions (pH=4,8), real D-values are lower than model values with pH=7.
2) Under acidified condition, at higher processing temperature (over 107° C) our experimental D-values are higher than that of the literature models, while at lower temperatures they are mixed.

General Conclusions

1) Light acidification to pH 4.8-5.0 can allow a reduction of harsh thermal treatments in the canning industry (improved quality keeping safety).
2) For risk mitigation in the canning food industry, a validation with *C. botulinum* should be performed (appropriate thermal resistance) instead of surrogates as *C. sporogenes* PA 3679 has shown a much lower thermal resistance than the usual one reported in the literature;
3) As thermal resistance is dependant of multiples factors : strain, pH, media and acid type, validation should be done with appropriate strains.
4) Those results showed that existing models are not fully useful under acidified conditions when they don't respect a linear reduction.
5) Research still need to be pursued to get full approval of mild thermal treatment in canned foods with intermediate pH (4.8-5.0) and to explain the behavior of certain strains when the acidification effect is overwhelmed by the temperature effect.

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