



# USE OF MICROBIOLOGICAL TOOLS FOR VALIDATION OF FOOD PROCESSES

LAURE ROGER  
UNILEVER R&D VLAARDINGEN, NL

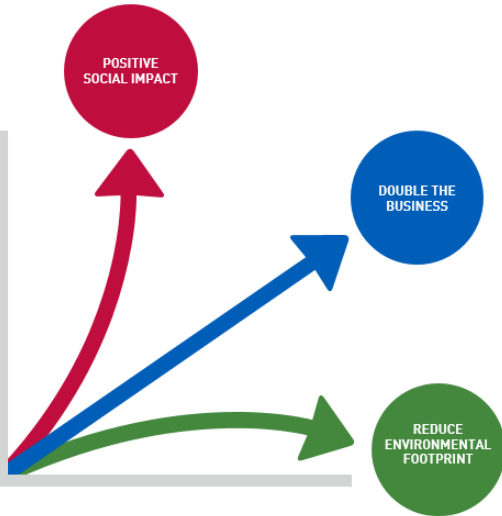
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# OVERVIEW



- Introduction
- A. Microbial Time Temperature Integrators (TTIs)
- B. New process validation
  - Ohmic line
- C. New packaging validation
  - Cold spots determination
- Concluding remarks

# BACKGROUND



**OUR VISION**  
TO DOUBLE THE SIZE OF OUR BUSINESS, WHILST REDUCING OUR ENVIRONMENTAL FOOTPRINT AND INCREASING OUR POSITIVE SOCIAL IMPACT

→ Ways to target an optimum process that can also prevent over-processing and high energy/water consumption

## MAKING PROGRESS 2010-2014

397 MILLION HELP TO IMPROVE HEALTH & HYGIENE

- 2% WATER
- 12% WASTE
- +4% GHG
- +21% UNDERLYING SALES GROWTH

238,000 WOMEN WITH ACCESS TO TRAINING & SKILLS

55% SUSTAINABLY SOURCED

800,000 SMALLHOLDERS HELPED & TRAINED

# INTRODUCTION



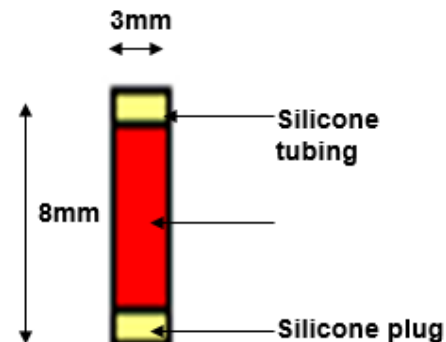
- Unilever has a diverse portfolio of products (>100 brands of food products)
- A multitude of core technologies are being used for production processes
- Innovation projects within R&D community
- Principles of microbiological hurdles



# INTRODUCTION

## Thermal processes

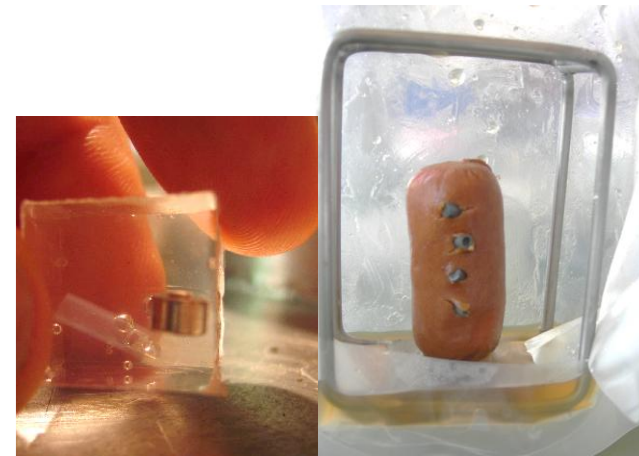
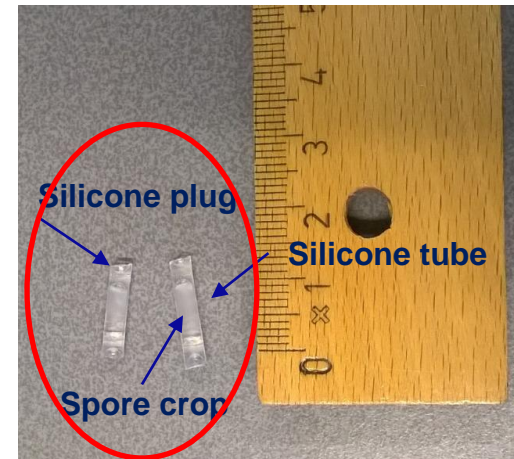
- Need tools to prove industrial thermal process is efficient
- Thermocouples allow measurements of time/temperature at a given place  
→ However, not always possible (continuous process, rotational process, presence of particles etc...)
- Introduction of use of biological markers such as previously isolated acid and/or heat-resistant bacterial spores/enzymes for validation of new processes/packaging
- In-house development of TTIs
  - Bacterial spores



# A. TIME TEMPERATURE INTEGRATORS (TTIs)



- TTI's can be defined as small measuring devices that show a time/temperature dependent irreversible change that mimics the change of a target attribute undergoing the same variable temperature exposure
- Incorporated within the food product (in liquid or in particles such as vegetables or meat)
- Examples of commonly used sporeformers: *Clostridium sporogenes* or *Bacillus stearothermophilus* strains  
→ here use of in-house isolated sporeformer strain



# A. BACILLUS SPOROTHERMODURANS IC4



- Isolation of a heat-resistant sporeformer: *Bacillus sporothermodurans* IC4 (van Zuijlen *et al.* 2010 *Food Research International*)
- IC4 was isolated from commercially sterilized Indian Curry soup in pillow bags, surviving the thermal process
- Due to high thermal resistance ( $D_{121^{\circ}\text{C}} > 5 \text{ min}$ )
  - was highly suitable for validating commercial low acid food thermal processes
- This strain is now used in validation studies in order to compare with F0 value predicted and measured
  - “Worst case” mesophilic sporeformer
- Characterisation of the *Bacillus sporothermodurans* IC4 strain

# A. BACILLUS SPOROTHERMODURANS IC4



- Specific equipment is needed in order to perform the thermal death time experiments (capillary tube technique or thermoresistometer)
- Keeps a stable heat resistance even after consecutive sporulation cycles
- Presence of a shoulder pattern in survival curves - as commonly observed with heat resistant spores
- Use of different non-log linear models to calculate “time to log reduction” in validation projects
- D and z values depends on sporulation conditions such as choice of media, ageing of the spores etc...

## Screenshot of the Time to log reduction model

LogN0 (log cfu/ml)

D121°C =	3.1323
Cc0 =	99.4489

T11 number	LogN (log cfu/ml)	Log Reduction	Time-To-LogReduction at 121°C
4	5.07	0.33	5.78

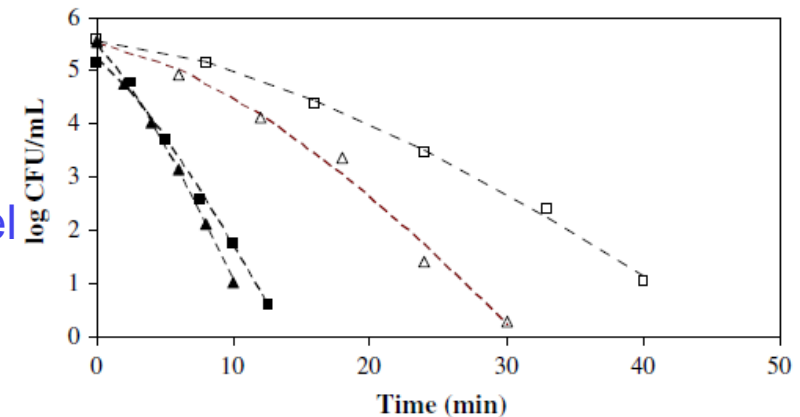


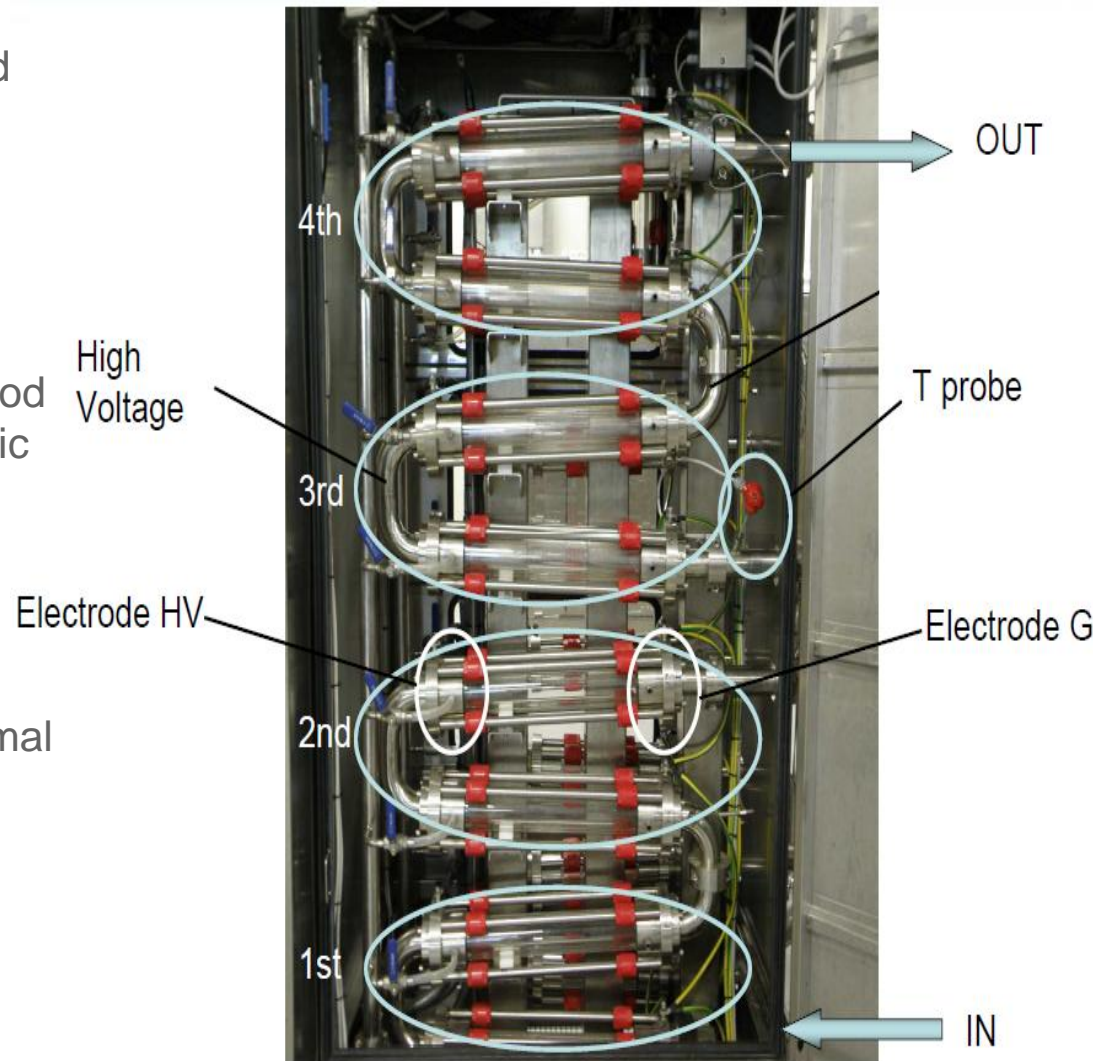
Fig. 2. Survival curves in distilled water of *B. sporothermodurans* using the capillary tube technique at 119 °C (□) or thermoresistometer at 119 °C (Δ) or with the capillary tube technique at 123 °C (■) or thermoresistometer at 123 °C (▲) fitted with Weibull model.



# B. NEW PROCESS - OHMIC LINE

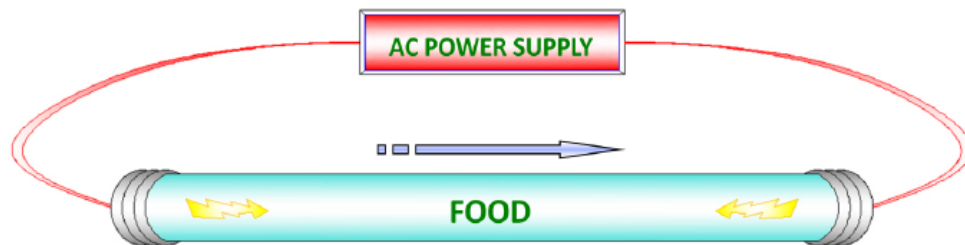


- Ohmic heating has ability to heat food rapidly and uniformly (even within particulates) – based on electrical conductivity
- No direct methods available for measuring the temperatures within food products undergoing continuous ohmic heating (not as easy as using a temperature probe)
- TTIs can be embedded within particulates to provide history of thermal process and measurement of the accumulated thermal lethality of process both in liquid and in particles



# B. NEW PROCESS VALIDATION – OHMIC LINE

- Ohmic line to sterilise continuously a low-acid food with particles
- Target microorganism: *Clostridium botulinum* ( $F_0 > 3$  min)
- Need to consider all types of worst case scenario so process usually much more severe than used for another validation type
- Determination:
  - ❖ coldest points (continuous flow)
  - ❖ residence time measurements (magnets)
  - ❖ conductivity
- ❖ In this process minimum  $F_0 > 6$  min needed (considering slowest heating particles and fastest flowing fraction)



# B. VALIDATION OF AN OHMIC LINE

- The following steps were carried out:
  - Design/validate/calibrate the line using the in house Thermal Integrator Model (“TIM”) model to obtain recorded process parameters
    - Set process parameters and predict resulting F-value
  - Determine conductivity according to size and consistency
  - Determine characteristics of TTIs
  - Preparation of TTIs in alginate particles
  - Process TTIs based on a processing setting calculated with the TIM model
  - Comparison of recorded F values and predicted F values
  - F values calculated from TTIs (time to log reduction)
  - Comparison of results of TTIs with calculations from TIM



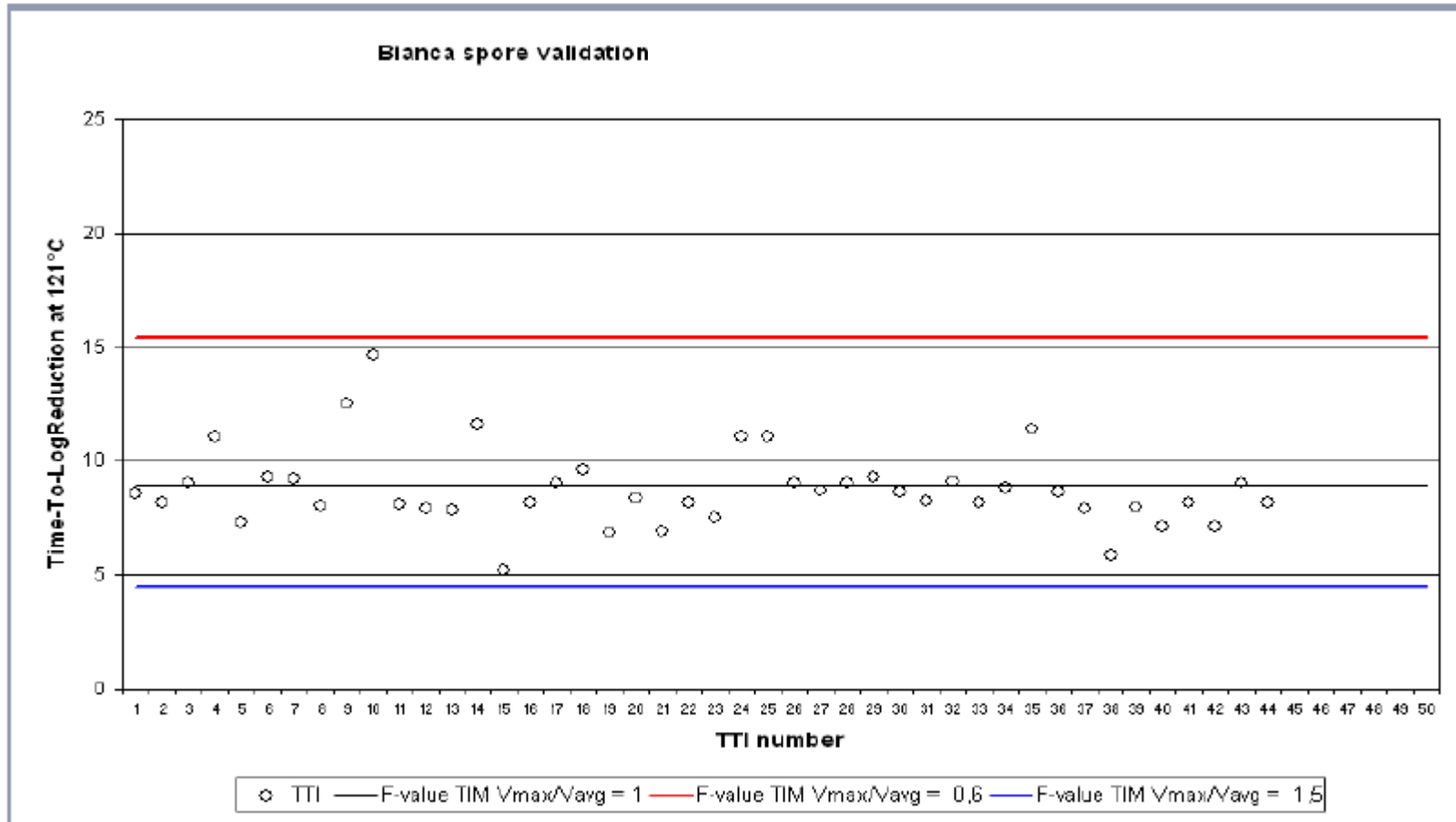
# B. SPORE TTI RESULTS OF OHMIC TRIAL



- F-values calculated from spore TTIs for an ohmically heated minestrone soup (Tref = 121.1°C, D121°C= 3.1 mins)
- Used ~ 50 TTIs per run

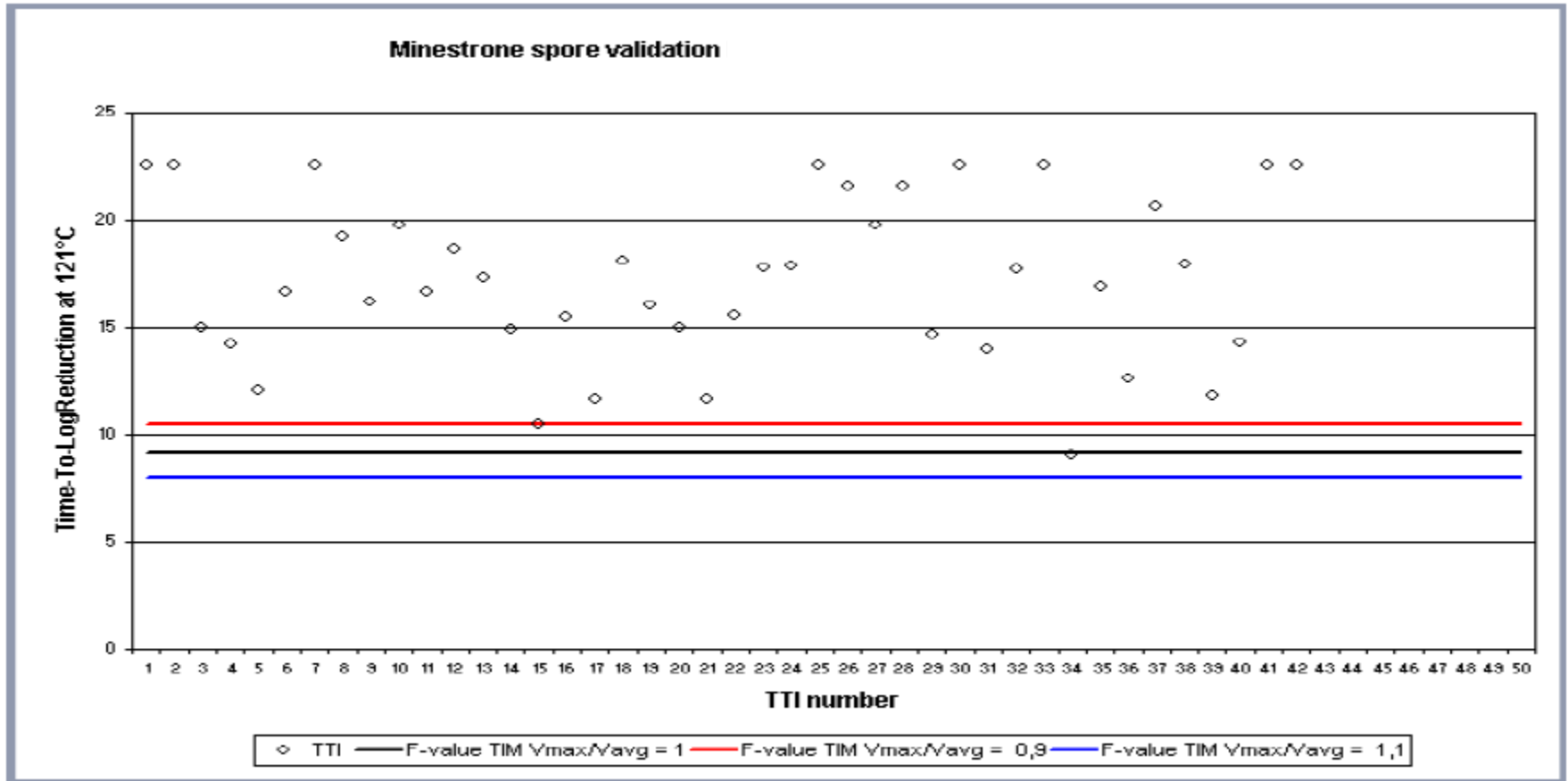
LogN0 (log cfu/ml)	<input type="text" value="7.2"/>			<input type="button" value="Clear Time-To-LogReduction"/>
D121°C =	<input type="text" value="3.1323"/>			<input type="button" value="Calculate Time-To-LogReduction"/>
Cc0 =	<input type="text" value="99.4489"/>			
TTI number	LogN (log cfu/ml)	Log Reduction	Time-To-LogReduction at 121°C	
1	2.00	5.20	22.56	
2	2.00	5.20	22.56	
3	4.42	2.78	14.97	
4	4.67	2.53	14.20	
5	5.35	1.85	12.06	
6	3.87	3.33	16.70	
7	2.00	5.20	22.56	
8	3.08	4.12	19.18	
9	4.03	3.17	16.21	
10	2.90	4.30	19.73	
11	3.87	3.33	16.70	
12	3.26	3.94	18.63	
13	3.68	3.52	17.29	
14	4.45	2.75	14.89	
15	5.84	1.36	10.47	
16	4.26	2.94	15.48	
17	5.48	1.72	11.64	
18	3.41	3.79	18.13	
19	4.09	3.11	16.00	
20	4.40	2.80	15.03	
21	5.47	1.73	11.67	
22	4.24	2.96	15.55	
23	3.53	3.67	17.76	
24	3.51	3.69	17.84	
25	2.00	5.20	22.56	
26	2.30	4.90	21.62	
27	2.90	4.30	19.73	

# B. COMPARING TTI VS. THERMOCOUPLES RESULTS



- Each dots is the actually F values achieved from this specific run
- The model give a good prediction according to the TTIs
- Model is fail-safe – to incorporate worst case

# B. COMPARING TTI VS. THERMOCOUPLES RESULTS



## B. PARTICLE SIZE AND CONDUCTIVITY

**Table 1. F-values calculated from Spore TTIs** ( $T_{ref} = 121.1^{\circ} \text{C}$ ,  $D_{121^{\circ} \text{C}} = 2.4 \text{ mins}$ )

particle type	Week 29-run 1	Week 29-run 2	Week 29-run 3
low salt/small	<b>12.9</b>	13.9	13.4
low salt/big	<b>&lt;6.6</b>	<b>&lt;6.6</b>	<b>&lt;6.6</b>
medium salt/small	15.5	>15.6	14.2
medium salt/big	13.5	14.5	14.2
high salt/small	>15.6	>15.6	>15.6
high salt/big	>15.6	>15.6	>15.6



- Salt concentration and size of particles have large impact on heat transfer
- Conductivity became a Critical Control Point

## B. SUMMARY OF OHMIC VALIDATION



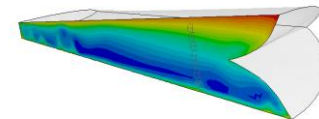
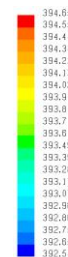
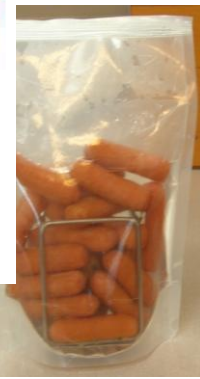
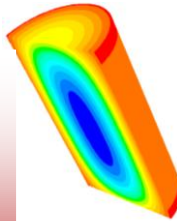
- Validation of the ohmic line was performed for these 2 very different soups (in terms of consistency and particulates)
- TTIs complemented temperature probes results
- Biological data had wider spread than predicted by TIM model and thermocouple records
  - Cooling rate of particles not considered in model (explains differences between TTIs and models)
- Conductivity and size of particles of food product crucial in setting the process



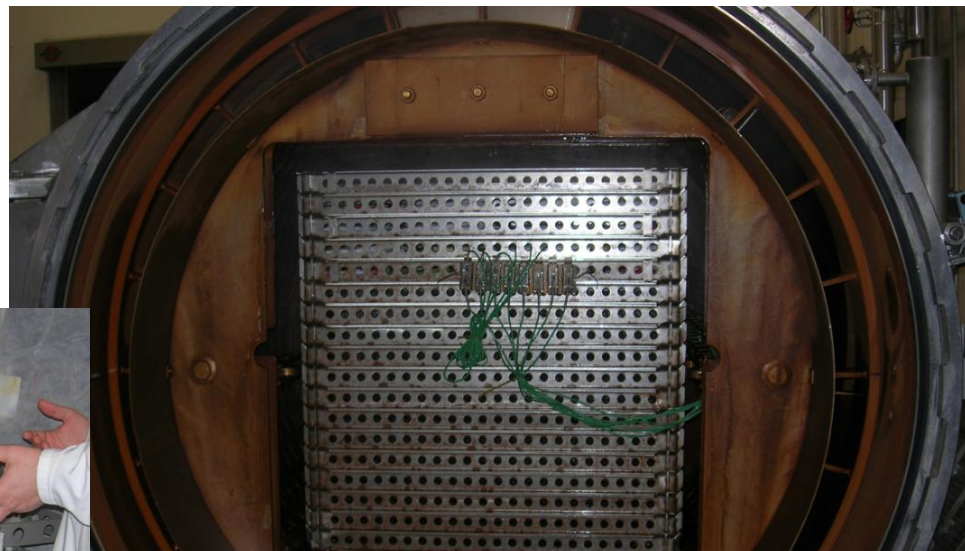
# C. NEW PACKAGING VALIDATION



- Changing from canned sausages with brine to a flexible pouch with and/or without brine
- Same recipe, but different packaging so need to consider:
  - Heat distribution in retort
  - Heat penetration into pouch
  - Presence and amount of gas/liquid
    - Convection (with brine) and conduction (no brine)
- Establishment of the required thermal process in order to reach  $F_0 > 5$  min at coldest slowest heating spot



# C. NEW DESIGN OF CRATES



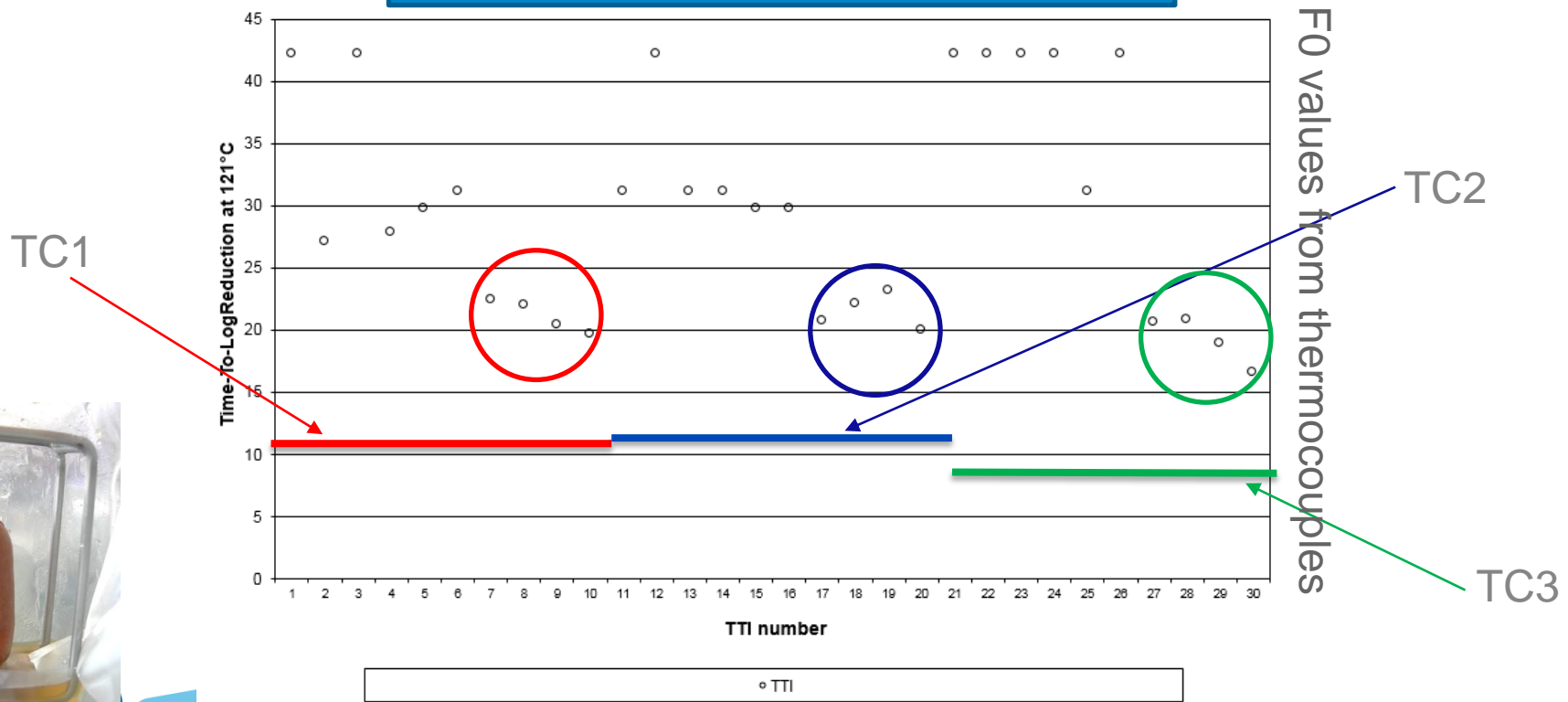
				Empty Layer
		TC11		Layer 20
				Layer 19
				Layer 18
		TC10		Layer 17
				Layer 16
TC9				Layer 15
				Layer 14
			TC8	Layer 13
		TC7		Layer 12
				Layer 11
TC6				Layer 10
				Layer 9
				Layer 8
				Layer 7
				Layer 6
		TC5		Layer 5
			TC4	Layer 4
		TC3		Layer 3
		TC2		Layer 2
TC1				Layer 1

FRONT VIEW

# C. NEW PACKAGING VALIDATION

- Latest experiment performed in 2015 in *B. sporothermodurans* IC4 (D (121°C) value = 4,8 min, z value = 8,3 °C)
- F0 target of 8-14 to achieve 2-3 log reduction

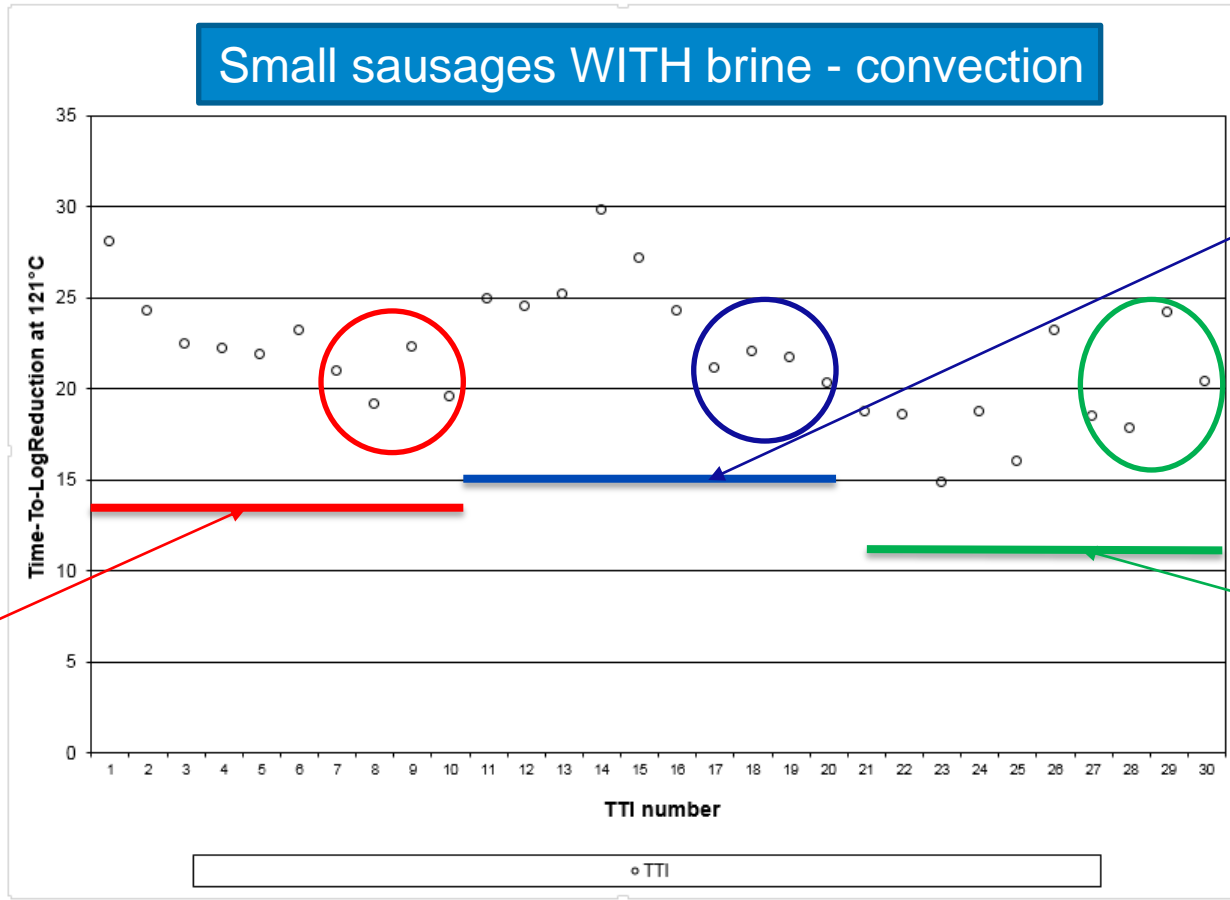
## Small sausages NO brine - conduction



# C. NEW PACKAGING VALIDATION



TC1



TC2

TC3

## C. SUMMARY CHANGE IN PACKAGING



- Mapping of retorts performed
- Determination of cold spot within retort
- Determination of cold spot within pouch
- TTIs showed that center of pouch not always coldest spot
- Statistical data analysis needs to be performed

# CONCLUDING REMARKS



- Use of microbiological tools within our Unilever factories
  - Microbial TTIs
  - New process + new packaging
- The use of microorganisms isolated from our own factories/products enables process validation closest to worst case scenario
- We maintain “out of ordinary” strains isolated from food within our culture collection for future trials and characterisation
- TTIs data complements measured and predicted data
- Comparing TTIs with thermocouple allows us to have a more complete picture of the new process
  - And in some cases the only way to understand a process
  - Biological variations (wider spread of data) needs to be taken into account

# Thank you



Email: [laure.roger@unilever.com](mailto:laure.roger@unilever.com)