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Risk assessment of *Salmonella* in Danish meatballs produced in the catering sector



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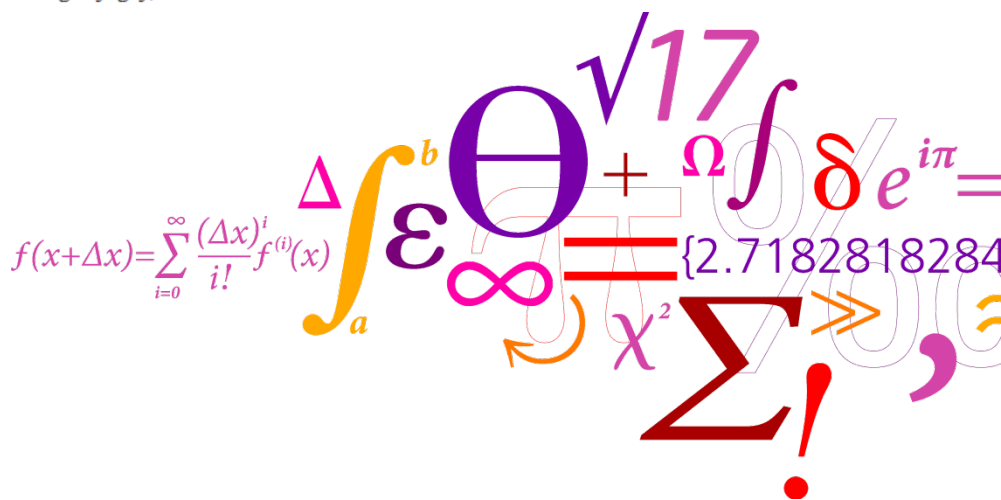
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DTU Food

National Food Institute



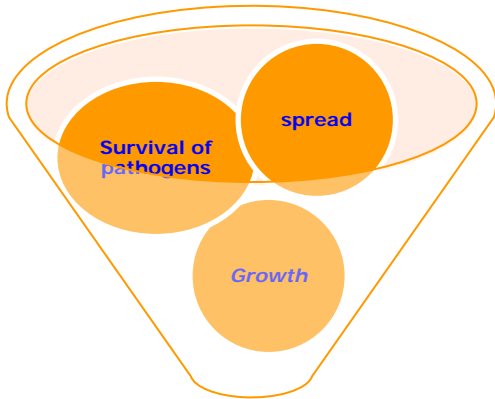
Outline:

- Introduction
- Objectives of the study
- Summarizing the performed work
- Process to build up the developed model
- Results
- Remarks and future perspectives

Introduction

- *Salmonella* is a critical pathogen (CDC, 2011; EFSA, 2010).
- Pork still is an important source of salmonellosis (EFSA, 2010; van Hoek *et al.*, 2012; Wegener *et al.*, 2003).
- Ground meat is frequently associated with outbreaks of salmonellosis (Stock and Stolle, 2001).
- Up to 70% of foodborne illnesses are estimated to be linked to catered food (Filion and Powell, 2011; Hensen *et al.*, 2006; Jones *et al.*, 2004; Lee and Middleton, 2003).
- In Denmark, 61 of 86 reported outbreaks in 2011 were associated with outside-the-home settings (anonymous, 2012).
- To model the distribution of pathogens during the processing operation are of major relevance to risk analysts (Flores, 2006).

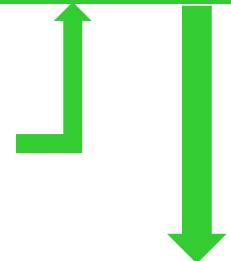
Objectives of the study



Challenging Food safety

Quantitative Microbiological Risk Assessment - QMRA

risk of salmonellosis by consumption of "frikadeller", processed by the catering sector



Raw pork

Final meal



Frikadelle

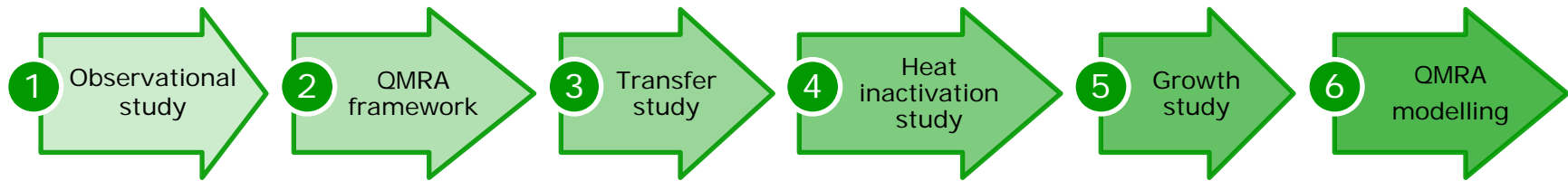
Catering processing

Based on scenario analysis:

- to evaluate existing practices
- challenge the efficiency of different Food Safety Authorities recommendations

Summarizing the performed work

Experimental work and modelling activities were performed:



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ORIGINAL ARTICLE

Modelling transfer of *Salmonella* Typhimurium DT104 during simulation of grinding of pork

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Effect of natural microbiota on growth of *Salmonella* spp. in fresh pork – A predictive microbiology approach

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^b Institute of Animal Science, University of Bonn, Katzenburgweg 7-9, 53115 Bonn, Germany

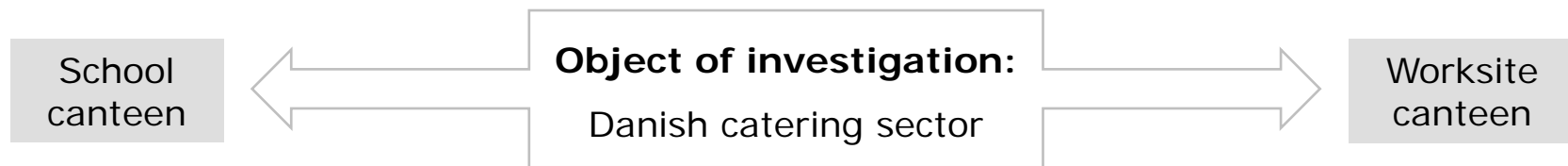
^c National Food Institute, Technical University of Denmark, Søtofts Plads, Building 221, DK-2800 Kgs. Lyngby, Denmark

Food microbiology

Volume 34, Issue 2, June 2013, pages: 284-295

1 Observational study

Experimental work

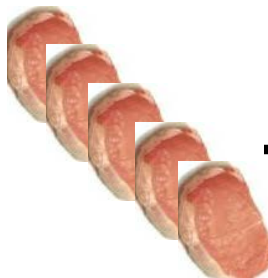


- Process flow
- Time and temperature profiles
- Weight of ingredients
- Unit changes during the processing, e.g.:

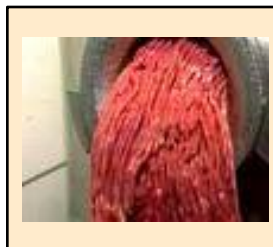
1 kg meat pieces



200 g slices



200 g portions



5 kg batter



70-80 g
"frikadelle" batter



2

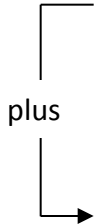
QMRA framework

Risk of salmonellosis from consumption of “frikadeller”

Modelling activities

Processing step	Basic activity	Source/model reference
Reception	1. initial contamination	Hansen et al. (2010)
Slicing	2. partitioning	Nauta (2005)
Grinding slices into portions	3. cross contamination	Møller et al. (2012)
Mixing of ingredients	4. mixing	Nauta (2005)
Dividing into meatballs	5. partitioning	Nauta (2005)
Heating inactivation in pan	6. inactivation	this study
Holding time	7. growth	Møller et al. (2013), this study
Heat inactivation in oven	8. inactivation	this study
Serving time plus cold storage until 6° C is reached	7. growth	Møller et al. (2013), this study
Estimation of the risk	9. dose response	FAO/WHO (2002)

Pathway



2

QMRA
framework

Risk of salmonellosis from consumption of 'frikadeller'

Processing step	Basic activity	Source/model reference
Reception	1. initial contamination	Hansen et al. (2010)
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Serving time plus cold storage until 6° C is reached	7. growth	Møller et al. (2013), this study
Estimation of the risk	9. dose response	FAO/WHO (2002)

plus

2 QMRA framework

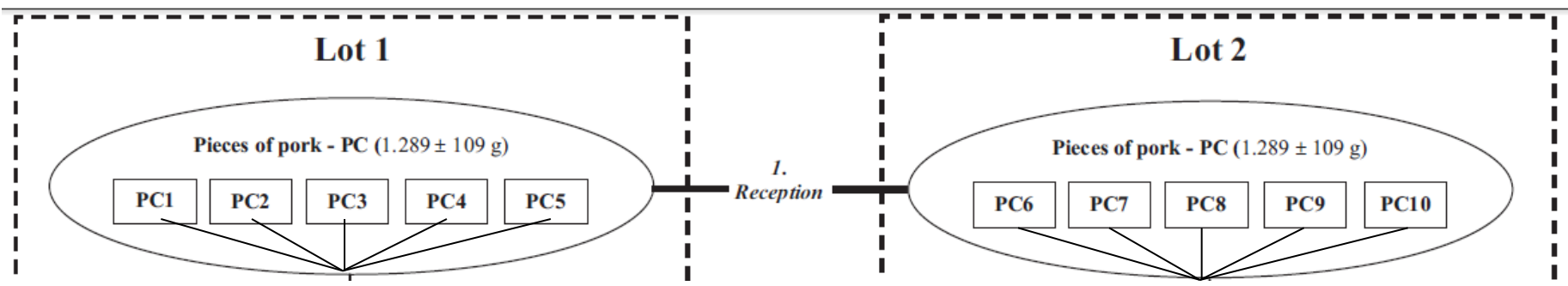
Risk of salmonellosis from consumption of 'frikadeller'

Processing step	Basic activity	Source/model reference
Reception	1. initial contamination	Hansen et al. (2010)

$R_{prevalence} = 4.2\%$ of the samples

- 39 positive samples within the interval 0.04–0.4 CFU/g
- 7 positive samples within the interval 0.4–4 CFU/g
- 5 positive samples within the interval 4–40 CFU/g
- 1 positive sample with more than 40 CFU/g (assumed max. 400 CFU/g)

~Histograma (log(0.04), log(400), {39,7,5,1})



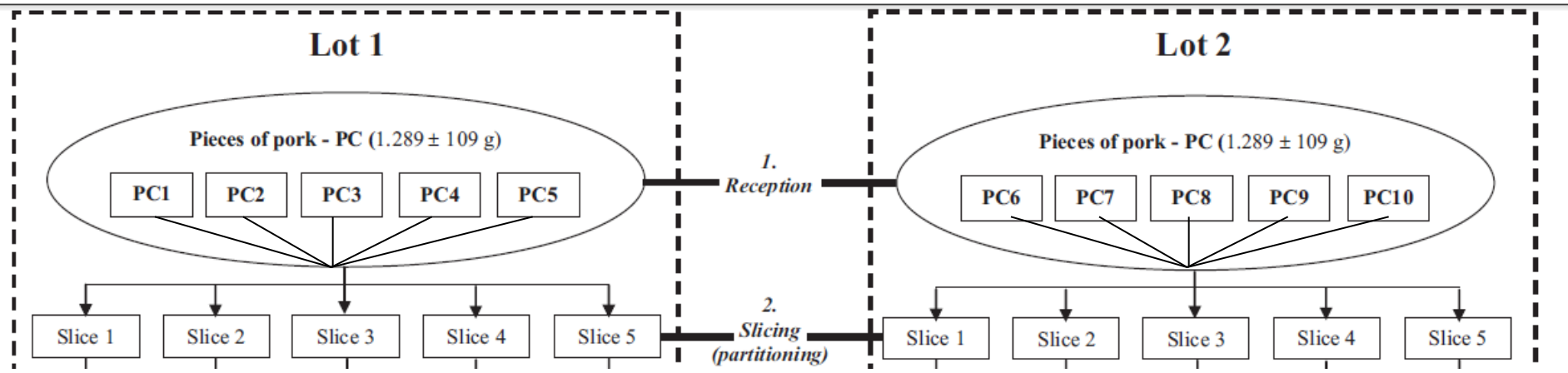
2

QMRA framework

Risk of salmonellosis from consumption of "frikadeller"

Processing step	Basic activity	Source/model reference
Reception	1. initial contamination	Hansen et al. (2010)
Slicing	2. partitioning	Nauta (2005)

$$S_{\text{conc slice}} \sim \text{Multinomial}(R_{\text{conc piece}}, \{1/S_n \text{ slices}\})$$



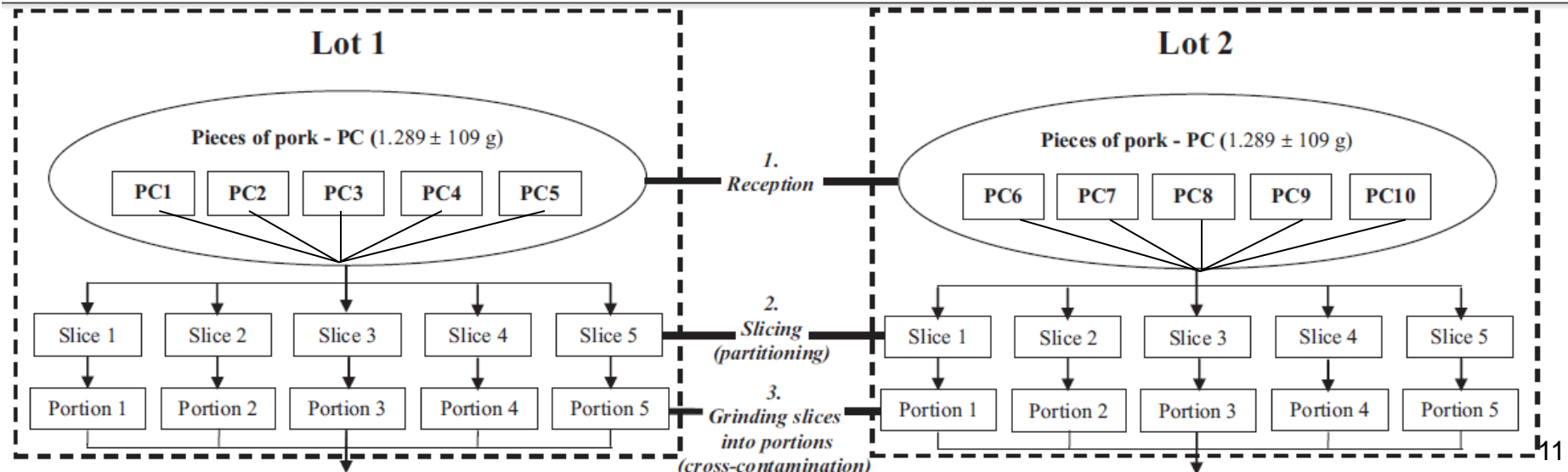
2

QMRA framework

Risk of salmonellosis from consumption of “frikadeller”

Processing step	Basic activity	Source/model reference
Reception	1. initial contamination	Hansen et al. (2010)
Slicing	2. partitioning	Nauta (2005)
Grinding slices into portions	3. cross contamination	Møller et al. (2012)

$$M_i = (1-a_1)(1-a_2)(1-c_2) S_i + (b_1 gr_{1,i-1}) + (b_2 gr_{2,i-1})$$

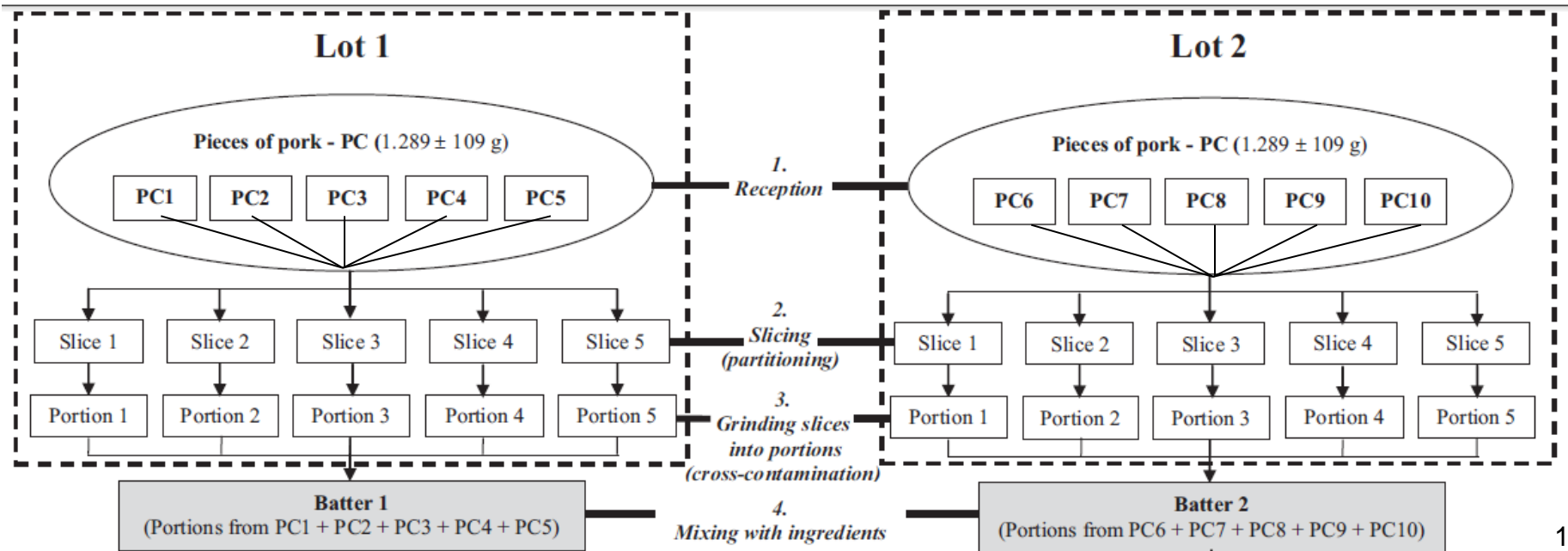


2 QMRA framework

Risk of salmonellosis from consumption of “frikadeller”

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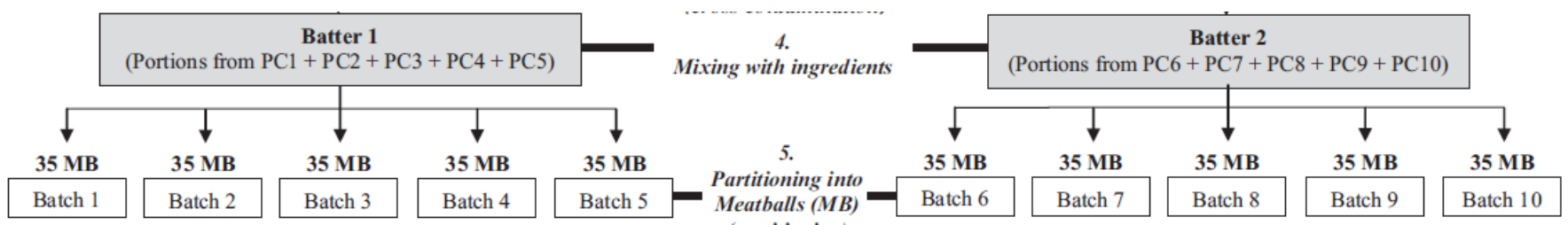
- the meat typically corresponds to 2/3 of the total weight,
- so the total weight is the weight of the meat multiplied by 3/2.



Risk of salmonellosis from consumption of "frikadeller"

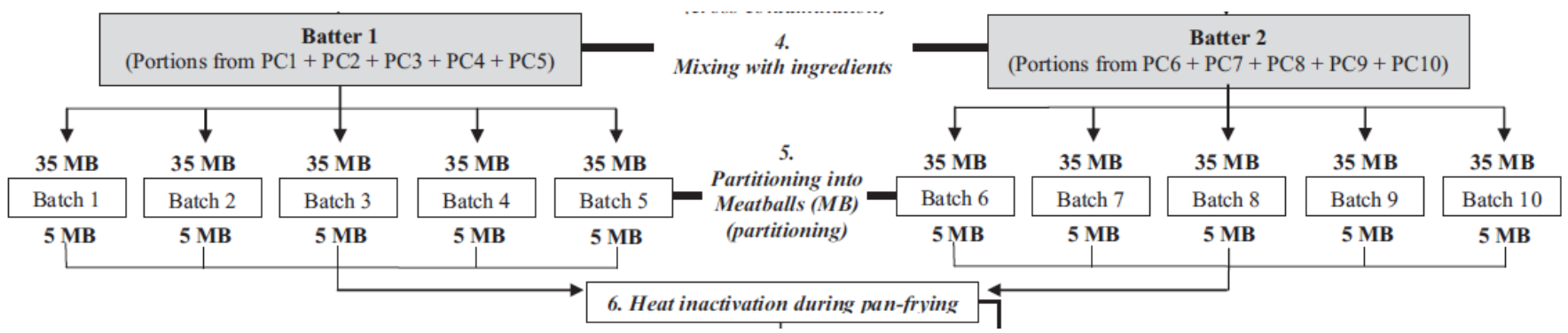
Processing step	Basic activity	Source/model reference
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Dividing into meatballs	5. partitioning	Nauta (2005)

~Multinomial(M_{conc} batter lot, $\{1/P_{weight}$ Meatball of P_n MB samples $\}$)



Risk of salmonellosis from consumption of "frikadeller"

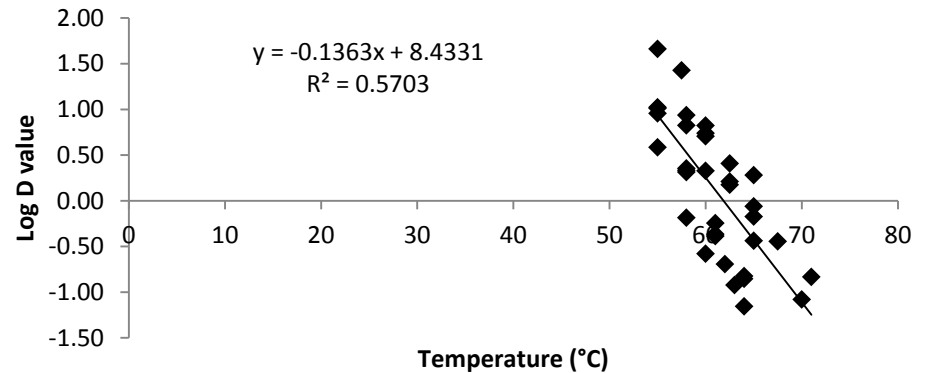
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Heating inactivation in pan	6. inactivation	this study



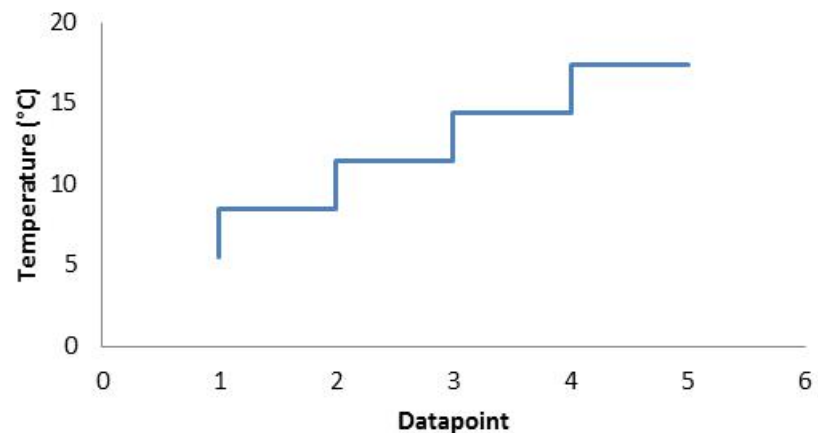
Heating inactivation in pan

Literature data	T (°C)	D (min)	log D
MURPHY et al., 2004. ground pork (40.2% fat)	55	45.87	1.66
	57.5	26.76	1.43
	60	5.07	0.71
	62.5	2.56	0.41
	65	1.91	0.28
JUNEJA et al., 2001 Pork (8.5% fat)	67.5	0.36	-0.44
	70	0.083	-1.08
	58	6.68	0.83
	60	6.65	0.83
JUNEJA et al., 2001 Beef (12.5% fat)	62.5	1.62	0.21
	65	0.87	-0.06
	58	8.65	0.94
SMITH et al., 2001. Low fat ground beef (4.8%)	60	5.48	0.74
	62.5	1.5	0.18
	65	0.67	-0.17
SMITH et al., 2001. Low fat ground beef (4.8%)	55	9.05	0.96
	58	2.26	0.35
	61	0.57	-0.24
SMITH et al., 2001. Low fat ground beef (4.8%)	64	0.15	-0.82
	55	10.55	1.02
	58	2.15	0.33
SMITH et al., 2001. Low fat ground beef (4.8%)	61	0.41	-0.39
	64	0.07	-1.15
	55	10.27	1.01
Velasquez et al. (2010) Ground pork (2.5% fat)	58	2.06	0.31
	61	0.43	-0.37
	64	0.14	-0.85
	55	3.846154	0.59
	58	0.653595	-0.18
JUNEJA et al., 2010 Lean ground beef	60	0.263158	-0.58
	62	0.20284	-0.69
	63	0.119474	-0.92
	60	2.12766	0.33
JUNEJA et al., 2010 Lean ground beef	65	0.364964	-0.44
	71	0.146628	-0.83

**Salmonella sp in ground pork and S. Typhimurium DT104
in low fat ground beef**



five datapoints in the heating curve



Heating inactivation in pan

Appendix B Observed data used to model heat inactivation of *Salmonella* during pan-frying.

Observed Batch	Start temperature ^a (°C)	Heating time (min)	End temperature (°C) per measured Meatball (MB)									
			MB 1	MB 2	MB 3	MB 4	MB 5	MB 6	MB 7	MB 8	MB 9	MB 10
2	5.1	7	19.9	23.5	31.9	34.2	39.1	39.5	39.8	42.7	44.5	56.3
3	5.1	7	18.2	18.3	20.1	20.6	27.0	28.3	29.5	41.0		
4	5.1	7	23.3	32.5	34.0	35.5	37.9	42.4	47.9	52.1	54.0	59.0
5	5.6	10	42.1	44.3	48.6	52.2	54.3	54.6				
6	7.2	10	37.2	46.0	49.1	50.1	62.2	66.8				
7	7.2	10	39.9	40.1	42.3	44.8	50	51.5				
8	8.4	6	32.1	33.1	39.1	53.2	55.2	59				
9	10.2	11	21.1	24.5	27.3	33.1	36.3	41.7	47			
10	10.2	5	45.1									



^a Measured in the batter.

Model approach

Temperature profile:

$$T(t) = T_o + (T_{end} - T_o) / t$$

$$EF_{Tref} = \sum_{start}^{end} 10^{\frac{T(t)-T_{ref}}{z}} \Delta t$$

$$LR_{pan-frying} = \frac{EF_{Tref}}{D_{Tref}}$$

Parameter values

$$T_o = 5-10^{\circ} C$$

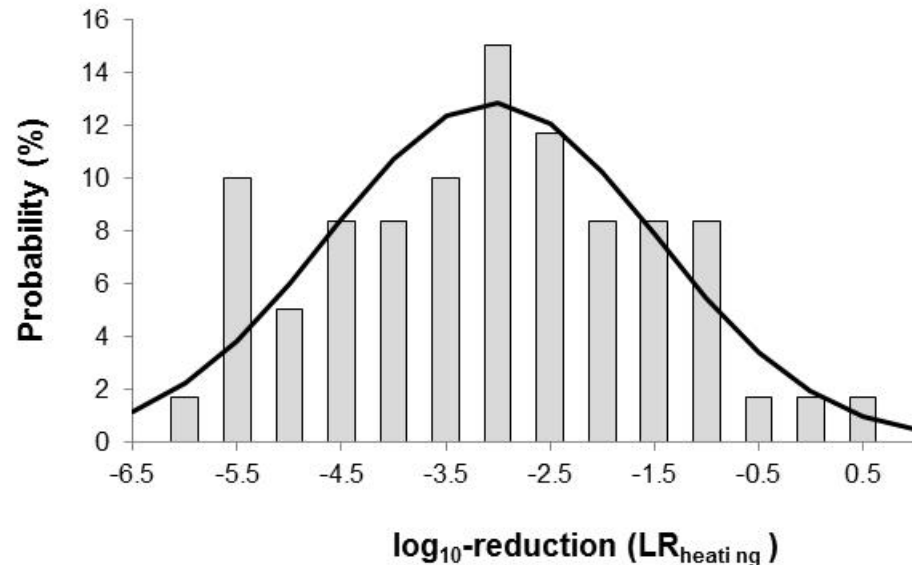
$$T_{end} = 18-67^{\circ} C$$

$$t = 7-11 \text{ min}$$

$$T_{ref} = 60^{\circ} C$$

$$z = 7.34^{\circ} C$$

$$D_{Tref} = 1.8 \text{ min}$$



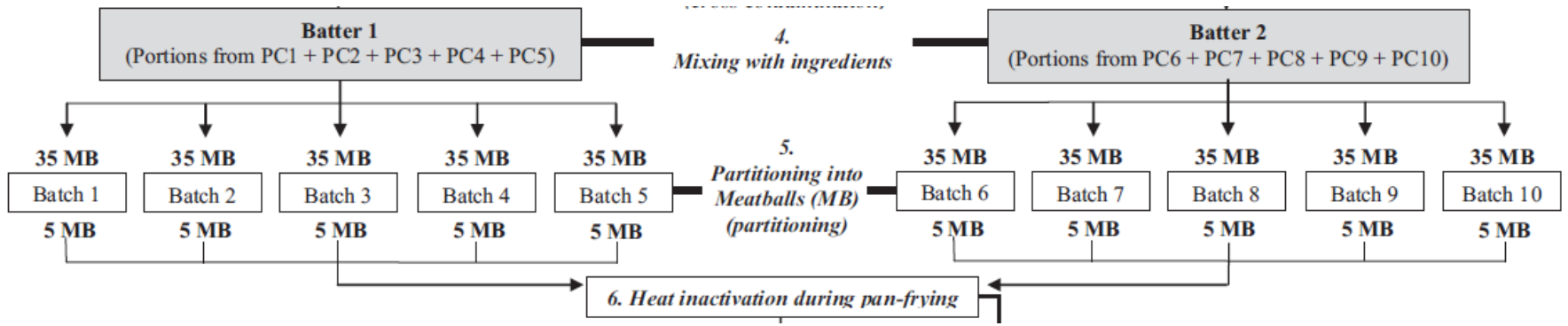
2 QMRA framework

Risk of salmonellosis from consumption of “frikadeller”

Processing step	Basic activity	Source/model reference
Reception	1. initial contamination	Hansen et al. (2010)
Slicing	2. partitioning	Nauta (2005)
Grinding slices into portions	3. cross contamination	Møller et al. (2012)
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Heating inactivation in pan 6. inactivation **this study**

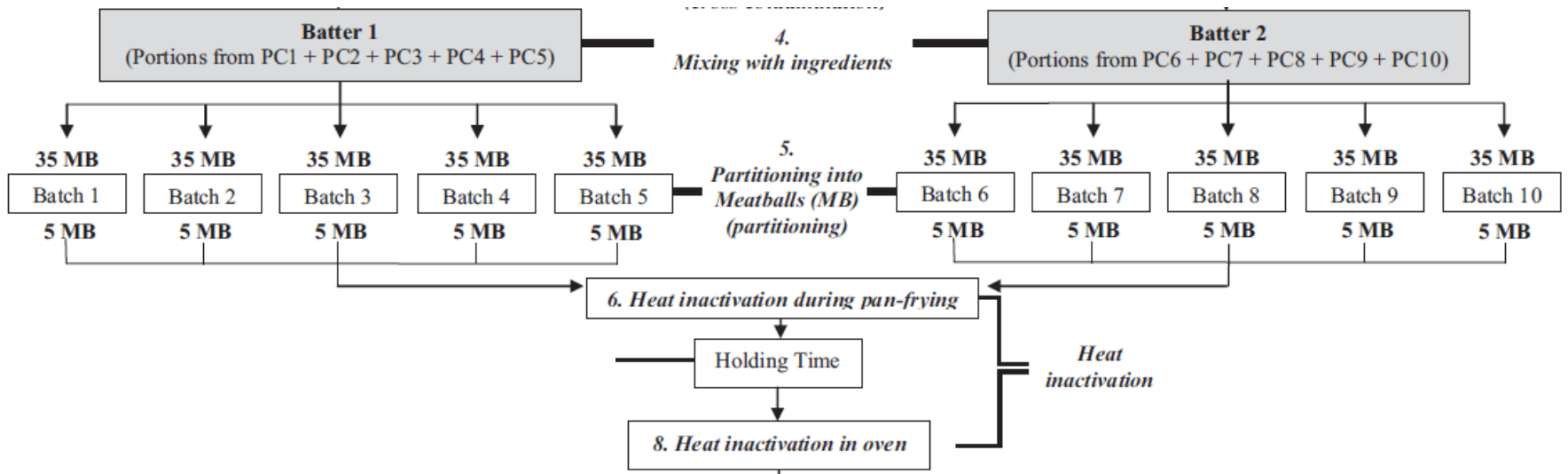
$$\sim \text{Poisson} (10^{(\log_{10}(\text{HP}_{\text{conc crit area}}))} - 10^{(\text{HP}_{\log \log \text{reduction}})})$$



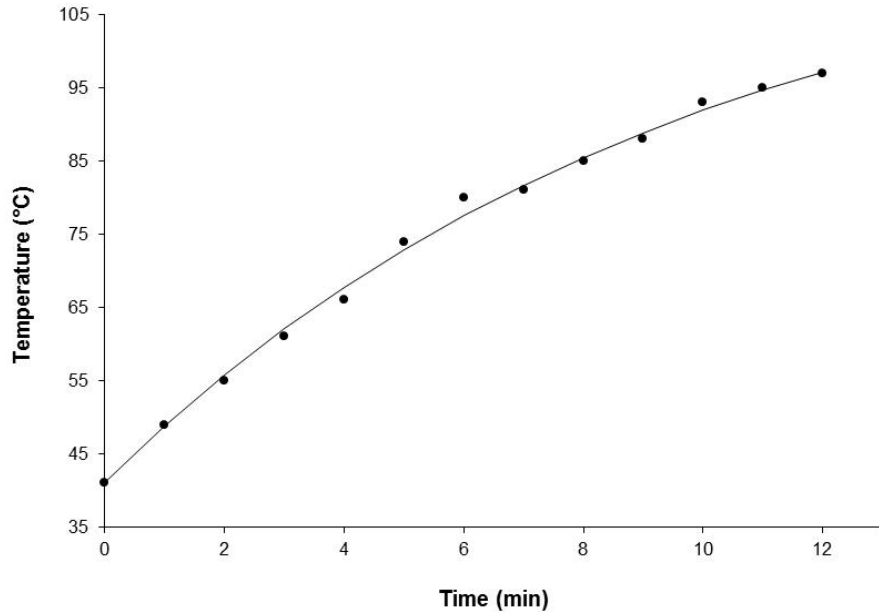
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Heating inactivation in pan	6. inactivation	this study
Holding time	7. growth	Møller et al. (2013), this study

Heat inactivation in oven 8. inactivation **this study**



Heating inactivation in oven



Model approach	Parameter values
Temperature profile:	$T_a = 121^\circ\text{C}$
$T(t) = T_a + (T_o - T_a) \cdot \exp(-k \cdot t)$	$T_o = 25-45^\circ\text{C}$
	$k = 6.1 \text{ h}^{-1}$
$EF_{Tref} = \Delta t$	$t = 4-11 \text{ min}$
$LR_{oven} = \frac{EF_{Tref}}{D_{Tref}}$	$T_{ref} = 65-75^\circ\text{C}$
	$z = 7.34^\circ\text{C}$
	$D_{Tref} = 0.4-0.02 \text{ min}$

Observed (●) and fitted with Newton's Law equation (—) temperature profiles of heating of meatball in oven until an end temperature of 95° C.

Data on eight meatballs used to determine the degree of heat inactivation in oven

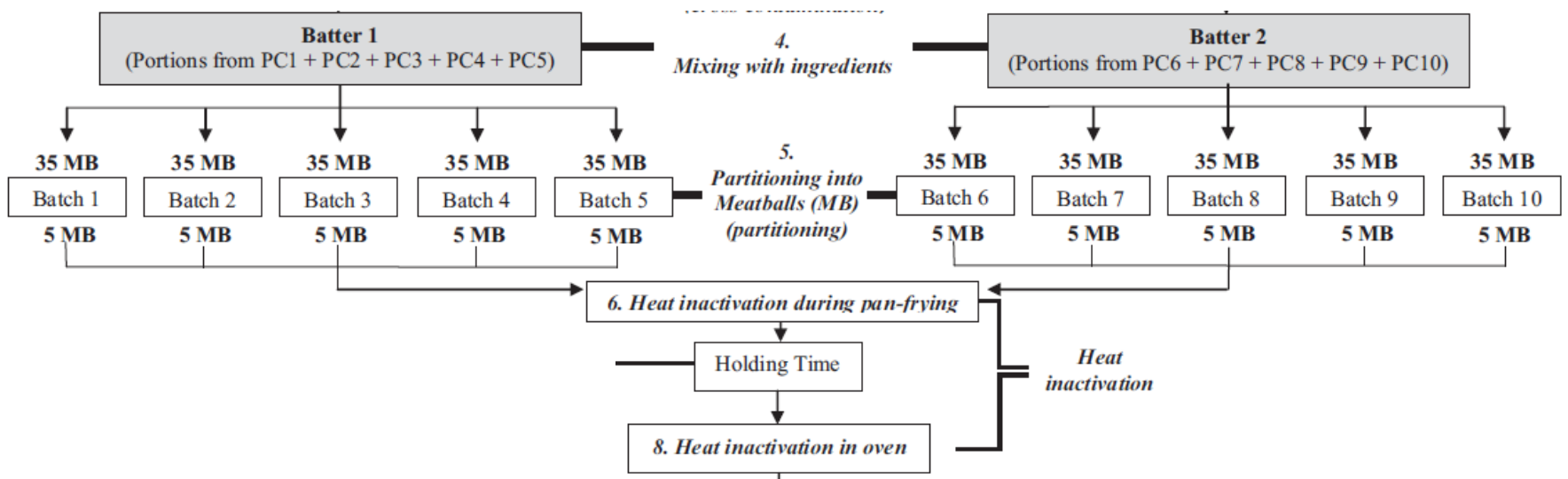
Observed meatball	Start temperature (T_o , ° C) ^a	Heating time (t, min) ^b	End temperature (° C) ^c	T_{ref} (° C)	Log (LR _{oven}) ^d
1	25.4	11.1	89.8	95	3.74
		5.4	65.8	75	0.25
		5.2	64.6	74	0.07
		5.0	63.4	73	-0.10
		4.8	62.2	72	-0.27
		4.6	61.0	71	-0.44
		4.4	59.8	70	-0.61
		3.5	53.8	65	-1.46

2 QMRA framework

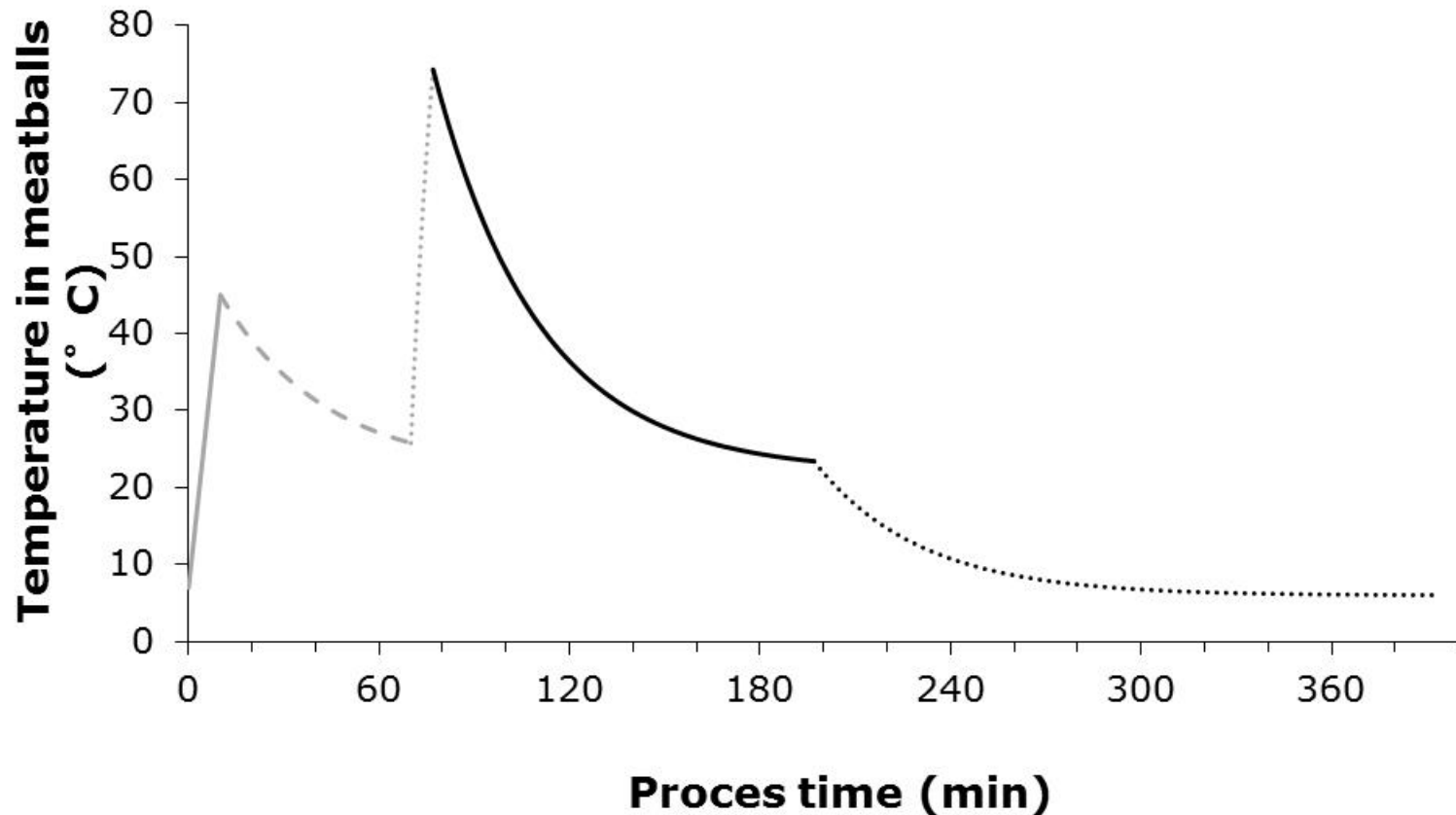
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Heat inactivation in oven	8. inactivation	this study

~ Normal (HO_{avg} log log red, $HO_{st dev}$ log log red)



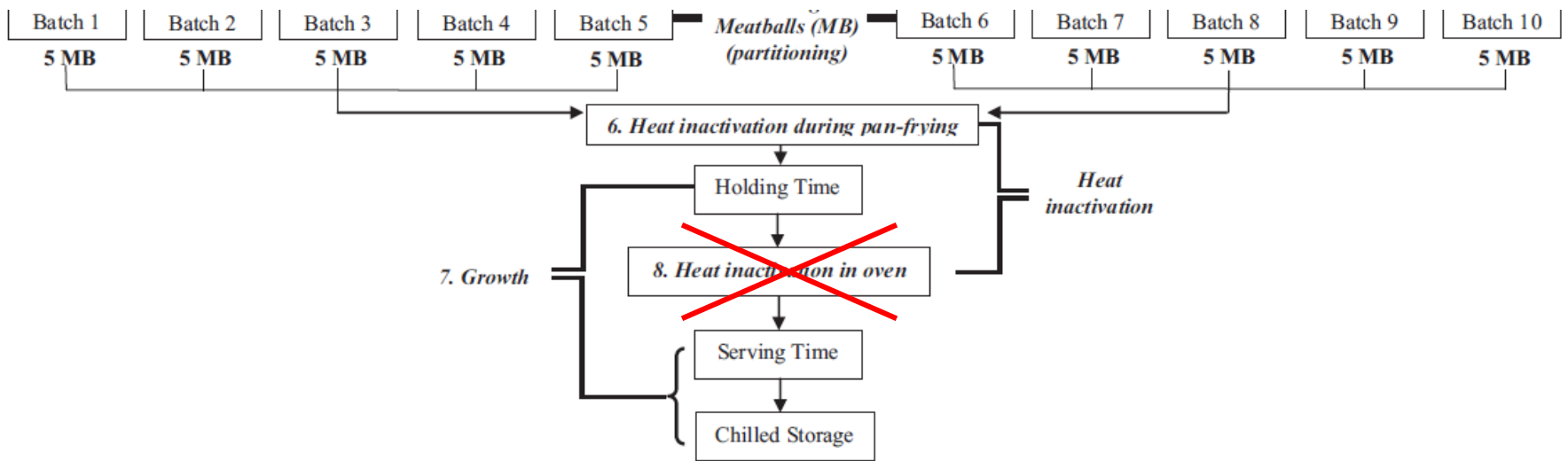
Example of temperature profile for a meatball during processing at the catering sector



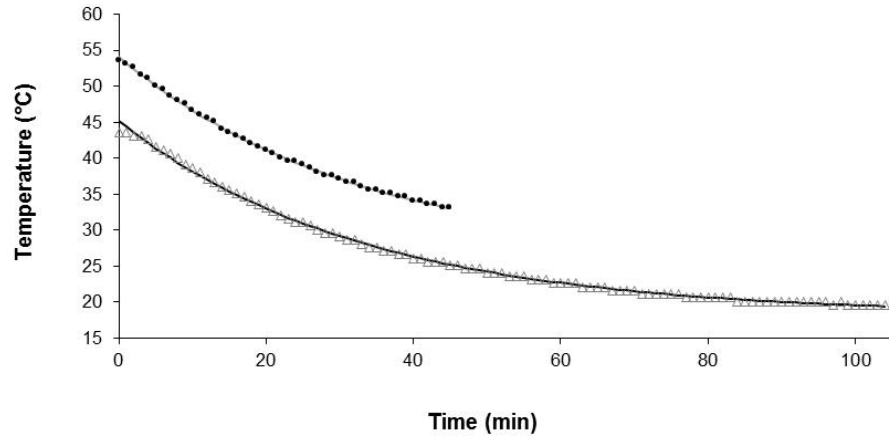
Involving: pan frying (grey full line), holding time (grey dashed line), heating in oven (grey dotted line), serving time (black full line), and chilled storage (black dotted line)

Risk of salmonellosis from consumption of "frikadeller"

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Dividing into meatballs	5. partitioning	Nauta (2005)
Heating inactivation in pan	6. inactivation	this study
plus Holding time	7. growth	Møller et al. (2013), this study
Heat inactivation in oven	8. inactivation	this study
plus Serving time plus cold storage until 6° C is reached	7. growth	Møller et al. (2013), this study



Growth during storage



Observed (•, Δ) and fitted (grey line, black line) temperature profiles of the cooling of meatballs in kitchens one and two, respectively. Fitting done with Newton's Law resulting in $k=1.8 \text{ h}^{-1}$ in both catering units.

Model approach

Temperature profile:

$$T(t) = T_a + (T_o - T_a) \cdot \exp(-k \cdot t)$$

$$\left. \begin{aligned} T_a &= 20\text{-}30^\circ\text{C} \\ T_o &= 18.2\text{-}66.8^\circ\text{C} \\ k &= 1.8 \text{ h}^{-1} \\ t &= 0\text{-}90 + 120 + 210 \text{ min} \end{aligned} \right\}$$

Growth model:

$$\mu_{max} = (b \cdot (T - T_{min}) \cdot (1 - \exp(c \cdot (T - T_{max}))))^2$$

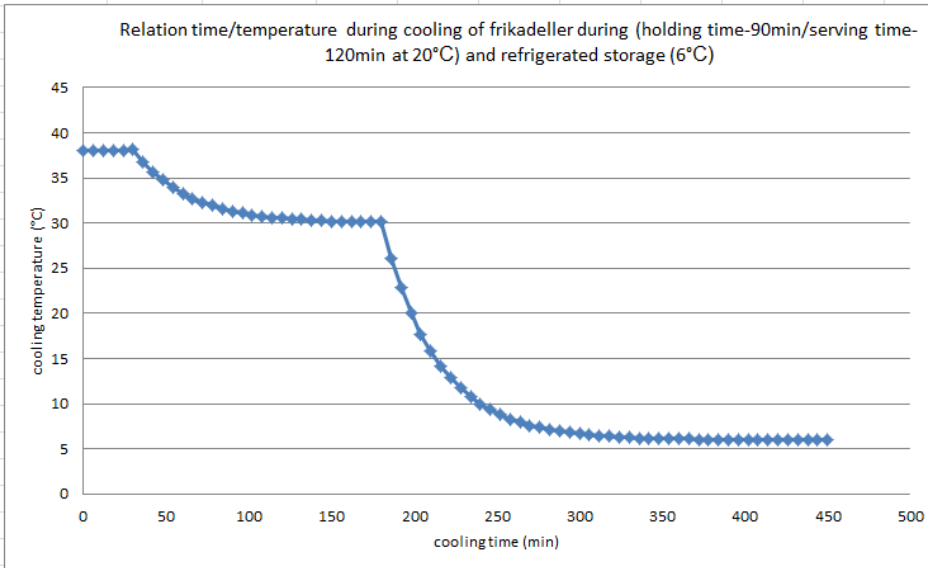
$$b = (0.04 \text{ h}^{-0.5} \cdot \text{C}^{-1}) \quad c = (0.43 \text{ C}^{-1})$$

T is temperature T_{min} (3.94°C) T_{max} (48.0°C)

$$\text{Lag time} = RLT \cdot \ln(2) / \mu_{max}$$

where RLT is the relative lag time of 3.10

cooling (min)	Temperatur	umax (1/h)	lag (h)	lag (min)	coolingtid minus le	le=ikke vækst ; 1=vækst
13	0	38	1.61246	1.3	80	0
14	6	38	1.61246	1.3	80	0
15	12	38	1.61246	1.3	80	0
16	18	38	1.61246	1.3	80	0
17	24	38	1.61246	1.3	80	0
18	30	38	1.62437	1.3	79	0
19	36	37	1.50509	1.4	86	0
20	42	36	1.40895	1.5	91	0
21	48	35	1.33107	1.6	97	0
22	54	34	1.26772	1.7	102	0
23	60	33	1.21599	1.8	106	0
24	66	33	1.17361	1.8	110	0
25	72	32	1.13879	1.9	113	0
26	78	32	1.11010	1.9	116	0
27	84	32	1.08642	2.0	118	0
28	90	31	1.06684	2.0	121	0
29	96	31	1.05062	2.0	123	0
30	102	31	1.03716	2.1	124	0
31	108	31	1.02599	2.1	125	0
32	114	31	1.01671	2.1	127	0
33	120	31	1.00899	2.1	128	0
34	126	30	1.00256	2.1	128	0
35	132	30	0.99720	2.2	129	1
36	138	30	0.99274	2.2	130	1
37	144	30	0.98903	2.2	130	1
38	150	30	0.98593	2.2	131	1



54 min. growth of Salmonella at 30C

6 min. growth of Salmonella at 26C

Growth during storage

Table 4. Change in log concentration $\Delta \log C_x$ (T_o , RTK, t_{holding}) for different start temperatures (T_o) observed after pan-frying, for different room temperatures in the kitchen (RTK) and holding times t_{holding} between 0 and 90 min followed by 120 min serving time and cold storage at 6°C.

RTK – Room Temperature in the Kitchen (°C)	T_o	$\Delta \log C_x$	Category ^a	$\Delta \log C_x$	Category
		(T_o , RTK, $t_{\text{holding}}=90$ min)		(T_o , RTK, $t_{\text{holding}}=0$ min)	
30	18.2	0.72	2	0.00	2
	32.0	0.96	2	0.24	2
	33.0	1.00	2	0.28	2
	40.2	1.13	2	0.41	2
	42.0	1.17	2	0.46	2
	43.0	1.18	2	0.46	2
	44.0	1.22	2	0.50	2
	45.0	1.22	2	0.50	2
	46.0	1.23	2	0.51	2
	47.0	1.18	2	0.46	2
	55.0	0.58	3 ^b	-0.14	3
	56.0	0.17	3	-0.33	3
	57.0	-0.04	3	-0.64	3
	58.0	-0.26	3	-0.98	3
	59.0	-0.84	3	-1.57	3
62.2	-3.64	3	-4.36	3	
25	18.2	0.19	2	0.00	1
	32.0	0.40	2	0.00	1
	40.2	0.59	2	0.09	2
	46.0	0.64	2	0.17	2
	52.0	0.47	3 ^b	-0.01	3
	55.0	0.14	3	-0.33	3
	57.0	-0.26	3	-0.74	3
	59.9	-1.04	3	-1.50	3
	62.2	-3.40	3	-3.85	3
20	18.2	0.00	1	0.00	1
	32.0	0.00	1	0.00	1
	35.5	0.02	2	0.00	1
	40.2	0.15	2	0.00	1
	46.0	0.19	2	0.00	1
	53.0	-0.01	3	-0.19	3 ^c
	55.0	-0.22	3	-0.38	3
	57.0	-0.58	3	-0.74	3
	59.0	-1.27	3	-1.44	3
	62.2	-3.43	3	-3.58	3

^a Process determining the result (see section 2.3.1.8). 1: lag phase; 2: growth; 3: inactivation.

^b Both growth and inactivation can occur within a time temperature profile. The data point is assigned to the category where it gives the best fit to the linear model.

^c No observations fall in category 2.

In the QMRA Monte Carlo
Normal distribution of T_o :

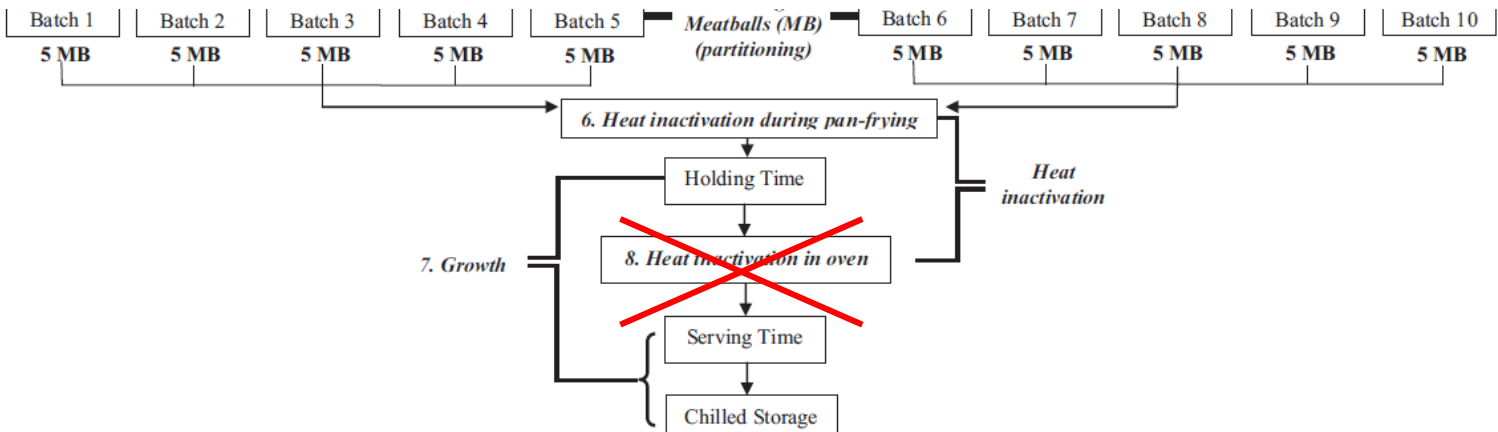
- mean 40.3
- standard deviation 12

Risk of salmonellosis from consumption of "frikadeller"

Processing step	Basic activity	Source/model reference
Reception	1. initial contamination	Hansen et al. (2010)
Slicing	2. partitioning	Nauta (2005)
Grinding slices into portions	3. cross contamination	Møller et al. (2012)
Mixing of ingredients	4. mixing	Nauta (2005)
Dividing into meatballs	5. partitioning	Nauta (2005)
Heating inactivation in pan	6. inactivation	this study

plus	Holding time	7. growth	Møller et al. (2013), this study
	Heat inactivation in oven	8. inactivation	this study
	Serving time plus cold storage until 6° C is reached	7. growth	Møller et al. (2013), this study

$$= (10^{(\log_{10}(HO_{\text{conc after inac}})} + 10^{(\Delta \log C_x))})$$



2

QMRA
framework

Risk of salmonellosis from consumption of “frikadeller”

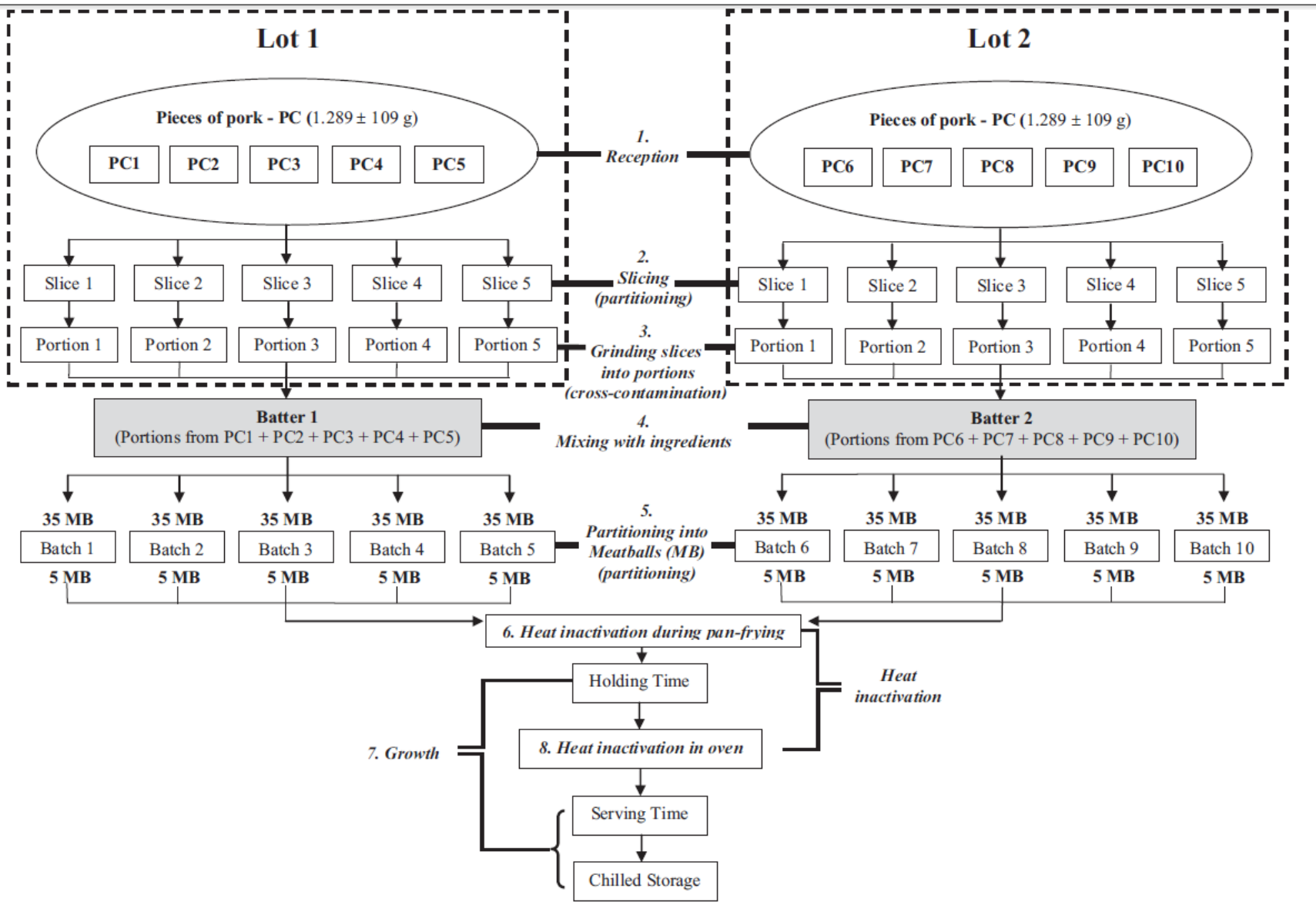
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Dividing into meatballs	5. partitioning	Nauta (2005)
Heating inactivation in pan	6. inactivation	this study
┌ Holding time	7. growth	Møller et al. (2013), this study
plus └ Heat inactivation in oven	8. inactivation	this study
└ Serving time plus cold storage until 6° C is reached	7. growth	Møller et al. (2013), this study
Estimation of the risk	9. dose response	FAO/WHO (2002)

$$Pill_{dose} \sim 1 - (1 + (C_{conc \text{ after } \Delta \log C_x} / \beta)^a$$

where $C_{conc \text{ after } \Delta \log C_x}$ is the concentration of *Salmonella* spp. in a meatball at the time of serving, a was -0.1324 and β equal to 51.45 (FAO/WHO, 2002).

The mean probability of illness from consumption of meatballs was used as the risk estimate

Risk of salmonellosis from consumption of 'frikadeller'



.1. Schematic presentation of processing for one lot of Danish meatballs (MB) at a catering unit. Grey boxes indicate the mix of ingredients into two individual batches of batter.

6

QMRA
modelling

Baseline model

Processing step	Parameters
Reception	Prevalence = 4.2%
	Concentration = 91 % 0.04-4 CFU/g 9 % → 400 CFU/g
Slicing	
Grinding slices into portions	
Mixing of ingredients	
Dividing into meatballs	
Heating inactivation in pan	
Holding time	
plus Heat inactivation in oven	75° C
plus Serving time plus cold storage until 6° C is reached	RTK = 20° C
Estimation of the risk	

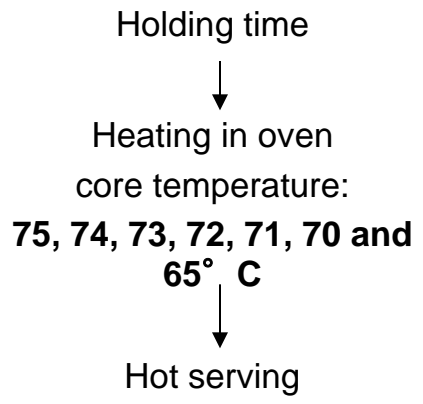
6

QMRA modelling

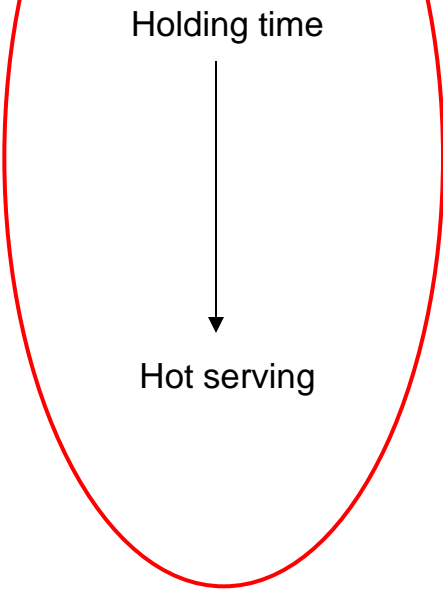
Scenario analysis

- Evaluate processing practices
- Test control measures

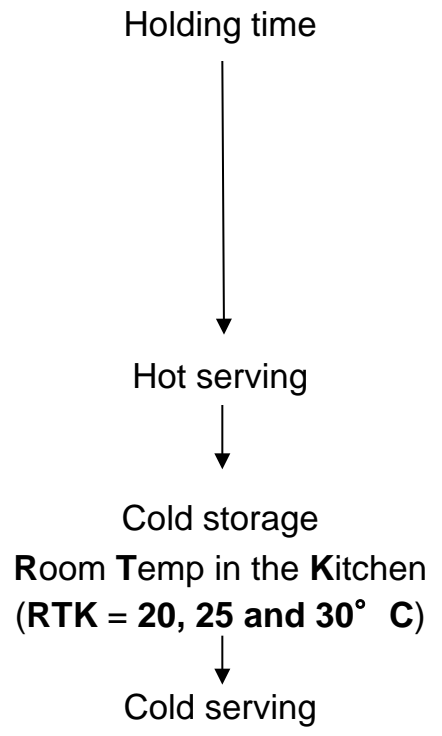
Scenario 1A – 1G



Scenario 2
Baseline scenario
RTK = 20° C



Scenario 3A – 3C



Scenario analysis

6

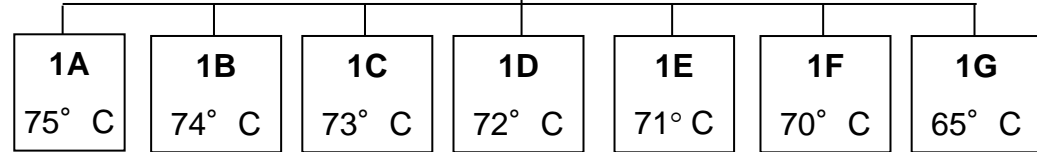
QMRA
modelling

Challenge 1:

Are the recommendations of Food Safety Authorities good enough?



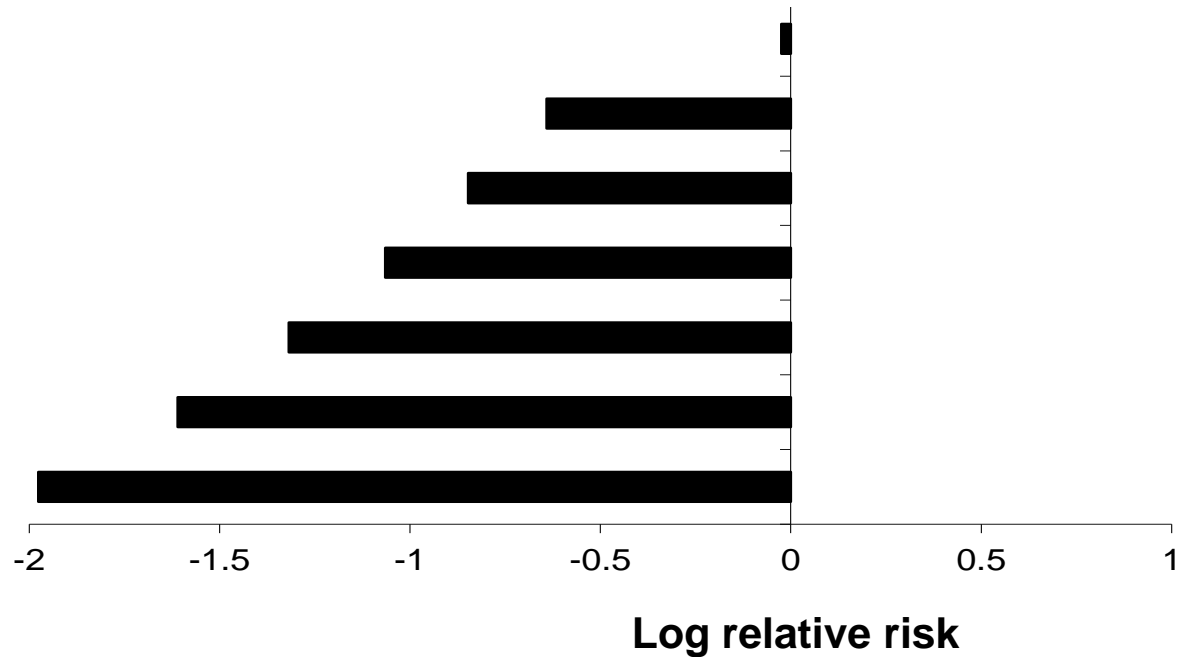
Core temperature of "frikadeller" in oven



Baseline scenario: no oven, RTK = 20° C
(1 case in 10,000 servings)

Tested scenarios

- 1G (heating in oven = 65°C)
- 1F (heating in oven = 70°C)
- 1E (heating in oven = 71°C)
- 1D (heating in oven = 72°C)
- 1C (heating in oven = 73°C)
- 1B (heating in oven = 74°C)
- 1A (heating in oven = 75°C)



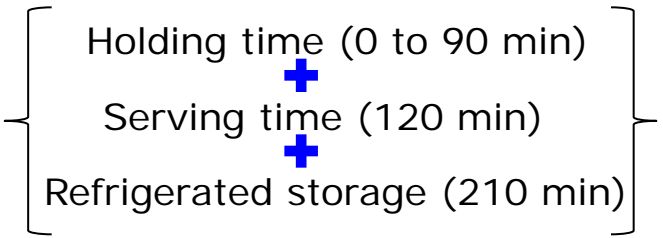
6

QMRA modelling

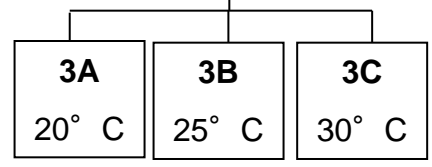
Scenario analysis

Challenge 2:

Is it safe to consume cold "frikadeller"?



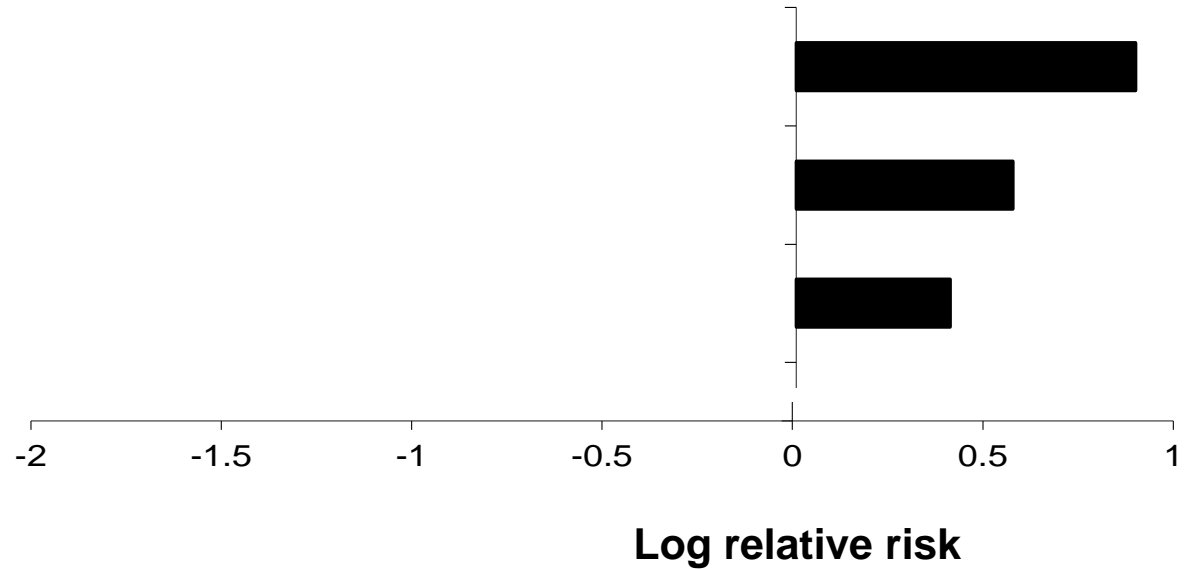
Room Temperature in the Kitchen (RTK)



Baseline scenario: no oven, RTK = 20° C
(1 case in 10,000 servings)

Tested scenarios

- 3C (no oven, rtk = 30°C)
- 3B (no oven, rtk = 25°C)
- 3A (no oven, rtk = 20°C)



6

QMRA
modelling

Scenario analysis

Challenge 3:

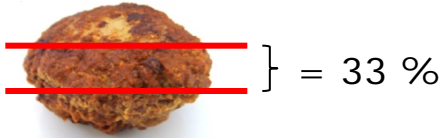
How many people would become ill with salmonellosis in Denmark per year?

serving times/year = 24		Danish population (2013) = 5600000		20% of Danish population = 1120000				
1A (heating in oven = 75°C)	1B (heating in oven = 74°C)	1C (heating in oven = 73°C)	1D (heating in oven = 72°C)	1E (heating in oven = 71°C)	1F (heating in oven = 70°C)	1G (heating in oven = 65°C)	2 (no oven nor storage)	3C (no oven, rtk = 30°C)
0.000005	0.000012	0.000027	0.000044	0.000081	0.000122	0.000541	0.001217	0.003332
						5.41E-04	1.22E-03	3.33E-03
137	332	717	1186	2168	3291	14556	32706	89562

6

QMRA modelling

Sensitivity analysis

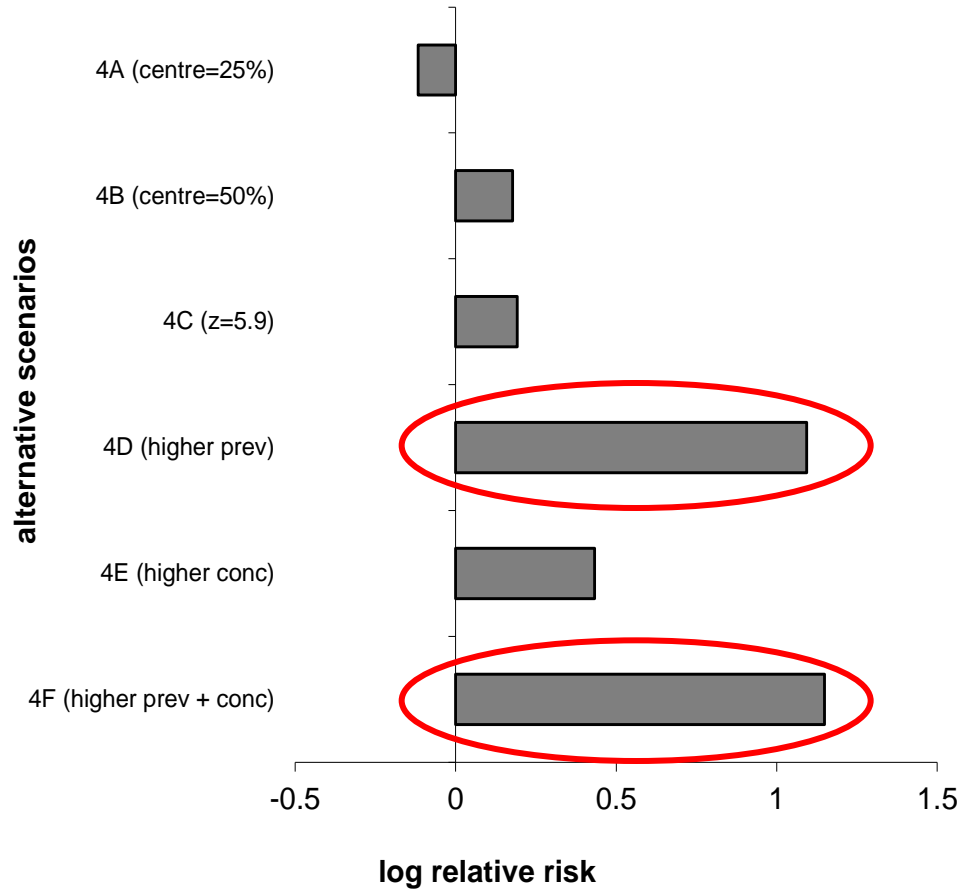
Investigated aspect	Baseline model	Sensitivity analysis	Alternative scenario
✓ Fraction of "frikadelle" with Survival of <i>Salmonella</i>	 = 33 %	<ul style="list-style-type: none"> = 25 % → 4A = 50 % → 4B 	
✓ Heating inactivation according to meat matrix	z-value = 7.34	z-value = 5.90	4C
✓ Prevalence of <i>Salmonella</i> in pork	4.2 %	20.0 %	4D
✓ Concentration of <i>Salmonella</i> in pork	91 % 0.04-4 CFU/g 9 % → 400 CFU/g	88 % 0.04-4 CFU/g 12 % 40-400 CFU/g	4E
✓ ↑ prevalence and ↑ concentration			4F

6

QMRA
modelling

Sensitivity analysis

scenario 1G, oven 65°C



↑ impact on the risk estimates



Combination of
↑ prevalence and ↑ concentration

Remarks and future perspectives

Risk assessment of *Salmonella* spp. in Danish meatballs produced at the catering sector

Cleide O. de A. Møller¹, Maarten J. Nauta¹, Donald W. Schaffner², Paw Dalgaard³, Bjarke B. Christensen^{1,4}, Tina B. Hansen¹

- ✓ The model flexible structure allows scenario analysis
- ✓ Core temperatures $> 70^{\circ}$ C inactivate *Salmonella*
- ✓ *No growth of Salmonella if:*
 - $RTK = < 20^{\circ}$ C
 - For 3.5 h until refrigeration
 - Refrigerated storage = $< 6^{\circ}$ C

To be investigated

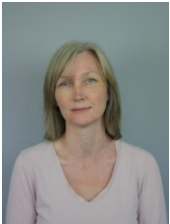
- ✓ Heat inactivation :
 - Specific heating profile
 - “Frikadelle” batter
- ✓ Growth
 - Heat injured *Salmonella* cells
- ✓ Improvement of the previously developed models (transfer and growth)



Acknowledgements

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Tina Beck Hansen



Maarten Nauta



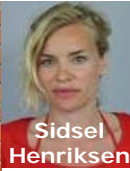
Donald W. Schaffner



Paw Dalgaard



Bjarke Bak Christensen



Sidsel Henriksen



Naseer Shukri



Birthe Hald

✓ Skillful technical assistance was provided by colleagues from the Division of Food Microbiology at the National Food Institute.



Rikke Krag



Mette Kemp



Kate Vibefeldt



Louise Vignæs