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

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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

Catering
processing

Final meal

Frikadelle

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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Food microbiology

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

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

Risk of salmonellosis from consumption of “frikadeller”

Modelling activities

Pathway

plus
→

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)

Risk of salmonellosis from consumption of 'frikadeller'

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

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)
 $R_{\text{prevalence}} = 4.2\%$ 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})

Lot 1

Pieces of pork - PC (1.289 ± 109 g)

PC1

PC2

PC3

PC4

PC5

1.
Reception

Lot 2

Pieces of pork - PC (1.289 ± 109 g)

PC6

PC7

PC8

PC9

PC10

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)

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

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)

$$M_i = (1-a_1)(1-a_2)(1-c_2) S_i + (b_1 \text{ gr}_{1,i-1}) + (b_2 \text{ gr}_{2,i-1})$$

Lot 1

Pieces of pork - PC (1.289 ± 109 g)

PC1

PC2

PC3

PC4

PC5

Slice 1

Slice 2

Slice 3

Slice 4

Slice 5

Portion 1

Portion 2

Portion 3

Portion 4

Portion 5

1.
Reception2.
Slicing
(partitioning)3.
Grinding slices
into portions
(cross-contamination)

Lot 2

Pieces of pork - PC (1.289 ± 109 g)

PC6

PC7

PC8

PC9

PC10

Slice 1

Slice 2

Slice 3

Slice 4

Slice 5

Portion 1

Portion 2

Portion 3

Portion 4

Portion 5

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)

- 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”

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Mixing of ingredients	4. mixing	Nauta (2005)
Dividing into meatballs	5. partitioning	Nauta (2005)

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

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

Heating inactivation in pan

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

= 33 %

Model approach

Temperature profile:

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

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

$$LR_{\text{pan-frying}} = \frac{EF_{T_{\text{ref}}}}{D_{T_{\text{ref}}}}$$

Parameter values

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

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

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

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

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

$$D_{T_{\text{ref}}} = 1.8 \text{ min}$$

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

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

Risk of salmonellosis from consumption of “frikadeller”

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Holding time	7. growth	Møller et al. (2013), this study
Heat inactivation in oven	8. inactivation	this study

Heating inactivation in oven

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

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

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

Risk of salmonellosis from consumption of “frikadeller”

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Holding time	7. growth	Møller et al. (2013), this study
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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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

Growth during storage

Model approach

Temperature profile:

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

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

Growth model:

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

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

$$T \text{ is temperature} \quad T_{min} (3.94^\circ \text{C}) \quad T_{max} (48.0^\circ \text{C})$$

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

where RLT is the relative lag time of 3.10

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

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

α 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'

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
→ Serving time plus cold storage until 6° C is reached	RTK = 20° C
Estimation of the risk	

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QMRA modelling

Scenario analysis

- Evaluate processing practices
- Test control measures

Scenario 1A – 1G

Scenario 2 Baseline scenario RTK = 20° C

Scenario 3A – 3C

6 QMRA modelling

Challenge 1:

Are the recommendations of Food Safety Authorities good enough?

Scenario analysis

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QMRA
modelling

Scenario analysis

Challenge 2:

Is it safe to
consume cold
“frikadeller”?

Holding time (0 to 90 min)

Serving time (120 min)

Refrigerated storage (210 min)

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)

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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 %	= 25 % = 50 %	4A 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

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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

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

To be investigated

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

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