SURVIVAL OF SPOROTHRIX SCHENCKII IN A MEAT PRODUCT OF ANIMAL ORIGIN

by

JOHN HOWARD SCHARDING
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Introduction

For the last 75 years the disease Sporotrichosis has appeared frequently in the world literature. Many aspects of this cutaneous and systemic fungal disease have been investigated, from the gross lesions in man and animals to the ultrastructure of the organism's cell wall. Hardly any body organ or tissue is exempt from invasion, and all common routes of entry are utilized by Sporothrix schenckii (6, 9).

Members of the <u>Sporothrix</u> (<u>Sporotrichum</u>) genus are known to survive at low temperatures since <u>Sporotrichum carnis</u> is responsible for the condition of "white spot" on chilled meats (2, 4), and <u>Sporothrix schenckii</u> was reported growing in and on frankfurters at 5 C (1). The organism's ability to survive at high temperatures was first reported in 1898, by Schenck (8). He noted that "the vitality of cultures is destroyed by exposure to a temperature of 60 C for five minutes." Hertoken and Perkins (5) found, in 1900, that 4 minutes at 60 C did not entirely kill <u>S. schenckii</u>, but that 4.5 minutes was fatal. De Beurmann (3) concluded in 1912, that the "spores can survive temperatures of 0 and 55 C plus." The methods utilized by these early researchers is not mentioned in their reports.

Despite all the literature on sporotrichosis, only one report suggests that this pathogenic fungus is capable of surviving in meat products, and this was in commercially processed frankfurters (1). The present study was undertaken to determine the extent to which <u>S. schenckii</u> can

survive the processing procedures currently employed in the manufacture of commercial frankfurters.

In order to accomplish this study, the processing times and temperatures had to be duplicated on frankfurters that were inoculated with the disease organism. Thus, a suitable frankfurter test model was devised which had the approximate length, diameter, and cooking characteristics of a commercial frankfurter.

Materials and Methods

Development of a test model

The frankfurter test model was fashioned from a 15.9 x 116 mm polypropylene test tube which had the walls of the lower portion of the tube partially removed. Over this basic model a section of cellulose casing was slipped and held in place with rubber bands. A glass test tube with a diameter slightly smaller than that of the model was inserted into the mouth of the model. This glass tube served as a plunger when stuffing the model with uncooked, inoculated frankfurter emulsion (Fig. 1). The entire assembled test model was autoclaved prior to each study, serving to initially sterilize the model and to shrink the cellulose casing for a more uniform fit around the glass tube insert.

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'Figure 1. Frankfurter test model: basic test model, cellulose casing, glass tube insert, and assembled test model.

Preparation of stock cultures

A human strain of <u>S. schenckii</u> (Culture B-958, Center for Disease Control, Atlanta, GA.) was maintained in both the mycelial and yeast phases on slants of Mycobiotic Agar (Difco Laboratories, Detroit, MI.). One agar slant of each phase was flooded with 5 ml of Brain Heart Infusion Broth (BHIB) (Baltimore Biological Laboratories, Baltimore, MD.) and harvested with a flamed inoculating needle. Each harvested culture was separately streaked onto several agar slants with a sterile swab. These two groups of stock cultures were allowed to grow for nine days in incubators at 25 C for the mycelial phase, and at 37 C for the yeast phase. At the end of this time, growth of the organism had achieved total coverage of the surface of the agar slants. All stock cultures were then placed in a refrigerator at 5 C until used in the experimental studies.

Inoculation of the emulsion

For each group of experimental studies approximately 120 g of uncooked, all-beef frankfurter emulsion (supplied by a Midwest producer) was blended in a sterile Waring Blendor with 25 to 50 ml of sterile distilled water. The addition of this small amount of water facilitated the filling of the test model with the emulsion. Two stock culture agar slants of either the mycelial or yeast phase were each flooded with 5 ml of BHIB and harvested with flamed inoculating needles. The entire 10 ml of harvested inoculum was added to the emulsion and thoroughly blended, yielding approximately 10 particles of <u>S. schenckii</u> per gram of emulsion, as determined by serial plate counting methods.

A sterile frankfurter test model was stuffed with the inoculated emulsion. A laboratory spatula and the glass tube insert were alternately used to fill and compress the emulsion inside the model and eliminate air pockets. Along with each study, another test model was filled with the inoculated emulsion and a dial thermometer inserted for recording the internal temperature of the emulsion during the cooking process. The models were then placed in a refrigerator at 5 C to equalize their beginning internal temperatures at 10 C prior to being simultaneously placed in an oven and the cooking process initiated.

Thermal processing of the emulsion

In personal communication, a large Midwest producer listed three reference points for the internal temperatures of their frankfurters using the following processing scheme: The starting internal temperature would be 10 C or less; at the end of 24 minutes at an oven temperature of 85 C, the internal temperature of the frankfurters would be about 54 C; and, after an additional 19 minutes at an oven temperature of 100 C, the final internal temperature of the frankfurters would be approximately 71 C. He further stated that few producers meet or exceed this 43 minutes, 71 C standard. Price and Schweigert (7) affirm this by stating that most producers achieve a final internal temperature in their frankfurters of 68 to 72 C. Thus, the cooking scheme outlined above, plus an additional seven minutes of processing time at 100 C, was adopted for the experimental studies. This gave a total experimental processing time of 50 minutes. A small, gravity convection laboratory oven (Blue M, model SW-11TA, Scientific Products, Evanston, IL.) was used as the cooking oven in these experimental studies.

Recovery procedures for S. schenckii

At periodic intervals during the cooking process, sterile swabs moistened with BHIB were inserted into the inoculated emulsion and streaked onto agar slants. Based on the results of pilot studies, the time intervals for attempting recovery of the organism were established at every four mintes from 0 to 20 mintes of processing time, every two minutes from 22 to 40 minutes, and at 43, 45, and 50 minutes. This made a total of 19 recovery attempts in each study. At each timed recovery attempt, the internal temperature of the emulsion was recorded. The recovery agar slants were incubated at 25 or 37 C, depending on the fungal phase being used, for seven days and the degree of growth of the organism was graded. Grading was as follows: O for no growth, +1 for 1 to 10 colonies, +2 for 11 to 20 colonies, +3 for 21 to 30 colonies, and +4 for more than 30 colonies. Sixty experimental studies were performed using the frankfurter test model and methods. Thirty of the studies used the mycelial phase stock cultures, and thirty the yeast phase stock cultures.

Results and Discussion

Test model

Table 1 lists the mean and standard deviation of the internal temperatures recorded for each processing time in the 60 experimental studies using the frankfurter test model, and Fig. 2 shows this data in graphical form.

Comparing the frankfurter test model internal temperatures with the temperature reference points for commercial frankfurters, the test model and experimental methods allowed close approximation to the processing conditions achieved in the manufacture of most commercial frankfurters. It is felt that the test model and methods represent a suitable procedure for the study of the survival of <u>S. schenckii</u> in frankfurters.

Experimental studies

In each study the final processing time, and the corresponding internal temperature, at which positive recovery of the organism was made using the frankfurter test model is shown in Table 2. For the total 60 studies, the mean of the final processing times for survival and recovery of <u>S. schenckii</u> was calculated to be 37 minutes, with a standard deviation of 7 minutes. The mean of the final internal temperatures was 67 C, with a standard deviation of 6 C. The range of final processing times was 26 to more than 50 minutes, and the range of final internal temperatures was from 51 to more than 77 C. Table 2 also indicates that in 32 % (19/60) of the experimental studies the final recovery times and internal temperatures met or exceeded the 43 minute, 71 C standard currently approximated by most commercial producers.

The number and percent of positive recoveries of <u>S. schenckii</u> from the frankfurter test model for each processing time in the 30 mycelial phase, 30 yeast phase, and 60 total studies are tabulated in Table 3. Figures 3 and 4 graphically display this data. Considering the percent recovery of the organism shown in Table 3 and Fig. 4, there was a

decrease from 98 % recovery at 26 minutes to 18 % at 38 minutes. This sharp decrease indicated the rapid death of the organism as time and temperature increased. However, there was still a 10 % positive recovery of <u>S. schenckii</u> after the full 50 minutes of experimental processing time. The slight increase, with a following decrease, in percent recovery at 38 to 40 minutes was due possibly to activation of dormant spores or to undetected experimental variation.

Appendix 1 lists the internal temperature and degree of recovered growth of the organism at each processing time for each experimental study.

It was implied in the hypothesis that only one source of contamination could occur in commercial frankfurters. This would be from undetected, diseased carcasses that were processed into various meat products. But, since this fungus is found worldwide on soil and organic matter (6), an additional source of both preprocessing and postprocessing contamination would be present wherever poor sanitary practices occur.

The results of these experimental studies indicate that <u>S. schenckii</u> can survive the processing procedures used in the manufacture of many commercial frankfurters to the extent that it could be a potential health hazard. Consideration of these findings is warranted by manufacturers, public health officials, and other responsible persons and agencies.

Table 1. Frankfurter test model internal temperatures for each processing time, based on 60 experimental studies.

D	T	Internal	temperatures (C)
Processing time (minutes) ¹	Temperature reference points (C) ²	Mean	Standard deviation
0	10	10	0
4		20	1
8		30	1
12		36	1
16		41	1
20		46	2
22		48	2
24	54	51	2
26		54	2
28		58	2
30		61	2
32		64	2
34		66	2
36		68	2
38		69	2
40		70	2
43	71	72	2
45		73	2
50		75	2

¹Oven temperatures of 85 C from 0 to 24 minutes, and 100 C from 26 to 50 minutes.

²For commercial frankfurters.

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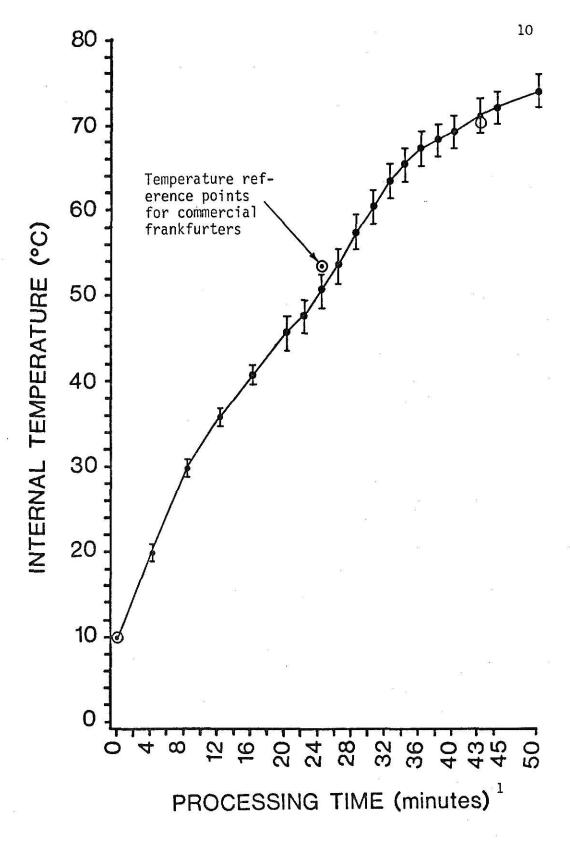


Figure 2. Mean and standard deviation of the frankfurter test model internal temperatures for each processing time, based on 60 l experimental studies. Oven temperatures of 85 C from 0 to 24 minutes, and 100 C from 26 to 50 minutes.

Table 2. Final processing times and internal temperatures for recovery of <u>S. schenckii</u> from the frankfurter test model.

30 Myceli	al phase	studies	30 Yeast phase studies
Exper- iment number	Final time (mins)	Final temp. (C)	Exper- Final Final iment time temp. number (mins) (C)
_			_
1m	45	69	5y 40 71
2m*	50	74	6y 36 68
3m*	45	75	7y* 43 71
4m	40	70	8y* 43 75
9m ×	43	74	13y* 45 76
10m	30	62	14y 36 66
11m*	43	75	15y* 43 74
12m	36	69	16y 36 67
17 m	34	64	23y 40 68
18m*	50	73	24y 30 60
19m*	50	77	25y 34 64
20m	40	72	26y 32 62
21m*	45	75	27y 40 70
22m*	50	77	28y 36 67
29m	30	63	35y 34 65
30m*	45	77	36y 34 65
31m	34	67	37y* 45 71
32m	32	64	38y* 50 74
33m	30	61	39y 32 66
34m	34	65	40y 26 55
41m*	43	73	47y 34 64
42m*	50	76	48y 26 51
43m	32	62	49y 30 60
44m	40	69	50y 28 56
45m*	43	71	5ly 30 60
46m	32	62	52y 32 63
53m	30	61	57y 30 63
54m	30	61	58y* 45 73
55m	28	59	59y 30 64
56m	36	68	60y 38 74
Mean**	39	69	36 66
Standard deviation	** 7	6	6 6

^{*}Indicates the 19 studies (32 %) that meet or exceed the 43 minute, 71 C standard.

^{**}Mean and standard deviation for all 60 studies is 37 \pm 7 minutes, and 67 \pm 6 C.

Table 3. Recovery of \underline{S} . schenckii from the frankfurter test model at each processing time.

Processing		al phase dies		phase dies	Combined studies			
time (minutes)	Num- ber	Per- cent	Num- ber	Per- cent	Num- ber	Per- cent		
0	30/30	100	30/30	100	60/60	100		
4	30/30	100	30/30	100	60/60	100		
8	30/30	100	30/30	100	60/60	100		
12	30/30	100	30/30	100	60/60	100		
16	30/30	100	30/30	100	60/60	100		
20	30/30	100	30/30	100	60/60	100		
22	30/30	100	30/30	100	60/60	100		
24	30/30	100	30/30	100	60/60	100		
26	30/30	100	29/30	97	59/60	98		
28	28/30	93	27/30	90	55/60	92		
30	25/30	83	19/30	63	44/60	73		
32	19/30	63	13/30	43	32/60	53		
34	14/30	47	11/30	37	25/60	42		
36	9/30	30	10/30	33	19/60	32		
38	8/30	27	3/30	10	11/60	18		
40	9/30	30	8/30	27	17/60	28		
43	7/30	23	3/30	10	10/60	17		
4 5	6/30	20	4/30	13	10/60	17		
50	5/30	17	1/30	3	6/60	10		

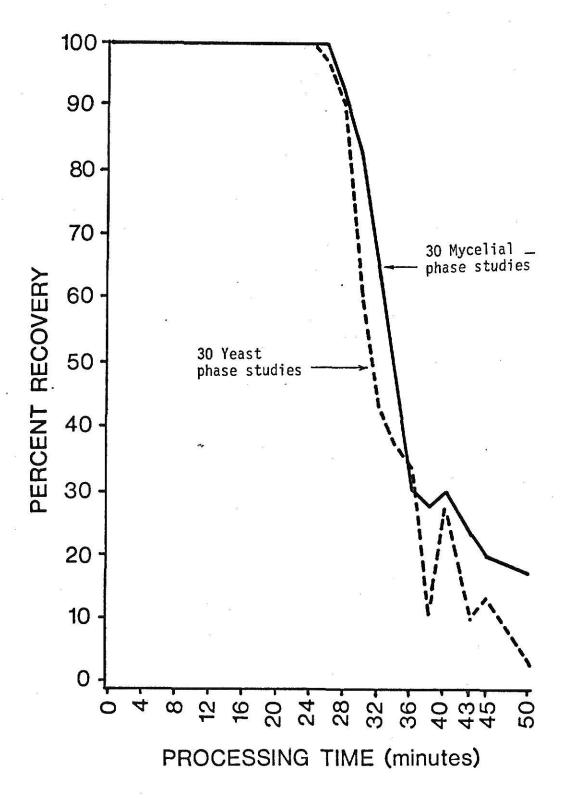


Figure 3. Percent recovery of <u>S. schenckii</u> from the frankfurter test model at each processing time for the mycelial and yeast phase studies.

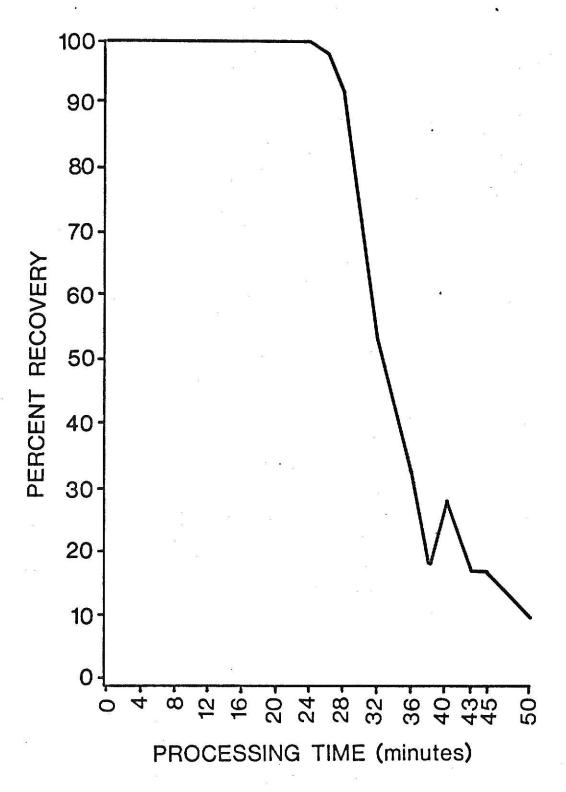


Figure 4. Percent recovery of <u>S. schenckii</u> from the frank-furter test model at each processing time for the combined 60 experimental studies.

References

- 1. Ahearn, D. G., and W. Kaplan. 1969. Occurance of <u>Sporotrichum</u> schenckii on a cold-stored meat product. Amer. J. Epidemiol. 89: 116-124.
- 2. Brooks, F. T., and C. G. Hansford. 1923. Mould growths upon cold-stored meat. Trans. Brit. Mycol. Soc. 8: 113-142.
 - 3. De Beurmann, L. 1912. On Sporotrichosis. Brit. Med. J. 2: 289-296.
- 4. Frazier, W. C. 1967. Food microbiology. 2nd ed., McGraw-Hill Book Co., New York, NY. p. 270.
- 5. Hertoken, L., and C. F. Perkins. 1900. Refractory subcutaneous abscesses caused by <u>Sporothrix schenckii</u>. A new pathogenic fungus.

 J. Exp. Med. 5: 77-89.
- 6. Lurie, H. I. 1971. Sporotrichosis, p. 614-675. <u>In</u> R. D. Baker (ed.), Human infection with fungi, actinomycetes and algae. Springer-Verlag, New York, NY.
- 7. Price, J. F., and B. S. Schweigert. 1971. The science of meat and meat products. 2nd ed., W. H. Freeman and Co., San Francisco, CA. p. 491.
- 8. Schenck, B. R. 1898. On refractory subcutaneous abscesses caused by a fungus possibly related to the Sporotrichia. Bull. John Hopk. Hosp. 9: 286-290.
- 9. Sethi, K. K. 1972. Experimental sporotrichosis in the normal and modified host. Sabouraudia 10: 66-73.

Appendix 1. Emulsion internal temperature (EIT) and degree of recovered growth of \underline{S} . schenckii (DRG) at each processing time for each experimental study.

All temperatures are in degree Centigrade (C).

Degree of recovered growth of \underline{S} . schenckii (DRG) graded as follows:

0 = no growth

+1 = 1 to 10 colonies

+2 = 11 to 20 colonies

+3 = 21 to 30 colonies

+4 = more than 30 colonies.

Summary of statistical data given on page 29.

Appendix 1. Emulsion internal temperature (EIT) and degree of recovered growth of \underline{S} . schenckii (DRG) at each processing time for each experimental study.*

Proces		1	m	2:	m	3	m	4	m	5	у
tin (minut		EIT	DRG	EIT	DRG	EIT	DRG	EIT	DRG	EIT	DRG
0	8	10	+4	10	+4	10	+4	10	+4	10	+4
4		22	+4	21	+4	20	+4	22	+4	21	+4
8		33	+4	32	+4	30	+4	30	+4	30	+4
12		39	+4	37	+4	36	+4	36	+4	36	+4
16		42	+4	42	+4	41	+4	40	+4	42	+4
20		45	+4	48	+4	47	+4	45	+4	47	+4
22	*	47	+4	51	+4	49	+4	47	+4	50	+4
24		48	+4	53	+4	52	+4	50	+4	52	+4
26		50	+4	56	+4	54	+4	53	+4	56	+4
28		55	+4	60	+4	59	+4	57	+4	60	+1
30		58	+4	63	+1	61	+1	60	+3	62	0
32		60	+2	66	0	64	+1	63	+1	64	0
34		62	+1	68	+2	66	+1	64	0	66	0
36		64	0	70	0	68	0	66	0	68	+1
38		66	0	70	0	70	+1	68	0	69	0
40		67	+1	71	0	72	0	70	+1	71	+1
43		68	0	72	0	74	+1	71	0	72	0
45		69	+1	73	0	75	+1	72	0	73	0
50		71	0	74	+4	76	0	74	0	75	0
Final	time:	45 m	ins.	50 m	ins.	45 m	ins.	40 m	ins.	40 m	ins.
Final	temp.:	69 C		74 C		75 C		70 C	YBSdamylow y a Berry on - is	71 C	

All temperatures are in Centigrade (C).

Appendix 1. Emulsion internal temperature (EIT) and degree of recovered growth of <u>S. schenckii</u> (DRG) at each processing time for each experimental study (continued).

Processing	6	У	7	1	8	y	91	m	1	10m	
time (minutes)	EIT	DRG	EIT	DRG	EIT	DRG	EIT	DRG	EIT	DRG	
0	10	+4	10	+4	10	+4	10	+4	10	+4	
4	21	+4	19	+4	20	+4	20	+4	20	+4	
8	30	+4	29	+4	30	+4	29	+4	30	+4	
12	37	+4	34	+4	35	+4	35	+4	35	+4	
16	42	+4	39	+4	41	+4	40	+4	42	+4	
20	47	+4	44	+4	47	+4	45	+4	47	+4	
22	49	+4	46	+4	50	+4	48	+4	50	+4	
24	51	+4	48	+3	53	+4	50	+4	52	+4	
26	55	+4	52	0	55	+4	53	+4	56	+ 4	
28	59	+1	55	+1	60	+1	59	+4	60	+1	
30	62	0	58	+1	63	0	62	+4	62	+1	
32	64	0	61	+1	65	0	64	+2	65	0	
34	66	0	63	0	68	0	67	+1	66	0	
36	68	+1	65	+1	70	0	69	+1	68	0	
38	69	0	67	0	72	0	70	+1	69	0	
40	71	0	69	+1	73	0	72	0	71	0	
43	72	0	71	+1	75	+1	74	+1	73	0	
45	73	0	72	0	7 5	0	75	0	74	0	
50	76	0	7 5	0	77	0	77	0	75	0	
Final time:	36 m	ins.	43 m:	ins.	43 m	ins.	43 mins.		30 mins.		
Final temp.:	68 C		71 C		75 C		74 C		62 C		

Appendix 1. Emulsion internal temperature (EIT) and degree of recovered growth of \underline{S} . schenckii (DRG) at each processing time for each experimental study (continued).

Proce		1	lm	1:	2m	1	3у	1.	4у	1	5у	
(minu		EIT	DRG	EIT	DRG	EIT	DRG	EIT	DRG	EIT	DRG	
0		10	+4	10	+4	10	+4	10	+4	10	+4	
4		21	+4	22	+4	22	+4	22	+4	22	+4	
8		30	+4	30	+4	32	+4	30	+4	31	+4	
12		37	+4	38	+4	38	+4	34	+4	37	+4	
16		44	+4	44	+4	42	+4	38	+4	42	+4	
20		49	+4	49	+4	47	+4	44	+4	48	+4	
22		52	+4	52	+4	50	+4	47	+4	51	+4	
24		54	+4	54	+4	52	+4	50	+4	53	+4	
26		57	+4	58	+4	56	+4	52	+4	56	+4	
28		60	+4	61	+4	60	+4	57	+1	60	+2	
30		63	+2	63	+1	63	+2	60	+1	63	+1	
32		66	+1	65	+1	66	+1	63	0	65	0	
34		69	+2	67	0	69	+1	65	+1	67	0	
36		71	+1	69	+1	71	0	66	+1	69	0	
38		72	0	70	0	72	0	67	0	71	0	
40		74	+1	71	0	73	+4	69	0	72	0	
43		75	+1	72	0	75	0	70	0	74	+1	
45		76	0	73	0	76	+1	73	0	75	0	
50		77	0	7 5 .	0	77	0	75 .	0	77	0	
Final	time:	43 mi	ins.	36 mi	36 mins.		45 mins.		36 mins.		43 mins.	
Final	temp.:	75 C		69 C	10 E1 9/8	76 C		66 C		74 C		

Appendix 1. Emulsion internal temperature (EIT) and degree of recovered growth of \underline{S} . schenckii (DRG) at each processing time for each experimental study (continued).

Process		1	бу	1	7m	1	Bm	.19	9m	2	Om
time		EIT	DRG	EIT	DRG,	EIT	DRG	EIT	DRG	EIT	DRG
0		10	+4	10	+4	10	+4	10	+4	10	+4
4		20	+4	19	+4	19	+4	20	+4	20	+4
8		28	+4	29	+4	29	+4	30	+4	30	+4
12		33	+4	35	+4	35	+4	37	+4	37	+4
16		38	+4	40	+4	40	+4	42	+4	42	+4
20	*	44	+4	45	+4	45	+4	48	+4	48	+4
22		47	+4	48	+4	48	+4	50	+4	50	+4
24	*	50	+4	50	+4	50	+4	53	+4	53	+4
26		52	+4	54	+4	54	+4	56	+4	56	+4
28		56	. +4 ,	57	+ 4	57	+4	61	+4	61	+2
30		60	+1	60	+4	60	+1	64	+4	64	0
32		63	+1	62	+1	62	0	66	+2	66	+1
34		65	+1	64	+3	64	0	68	+1	68	0
36		67	+1	66	0	66	0 -	70	+1	70	0
38		69	0	68	0	68	0	71	+1	71	0
40		70	0	69	0	69	0	72	+2	72	+1
43		71	0	70	0	70	0	74	0	74	0
45		72	0	71	0	71	0	75	0	75	0
50		74	0	73	0	73	+1	77	+1	77	0
Final t	time:	36 m	ins.	34 m:	ins.	50 mins.		50 mins.		40 mins.	
Final t	temp.:	67 C		64 C		73 C		77 C		72 C	

Appendix 1. Emulsion internal temperature (EIT) and degree of recovered growth of \underline{S} . schenckii (DRG) at each processing time for each experimental study (continued).

Processin	g	21m	2	2m	2	Зу	2	4y	2	5y
time (minutes)	EIT	DRG	EIT	DRG	EIT	DRG	EIT	DRG	EIT	DRG
0	10	+4	10	+4	10	+4	10	+4	10	1 4
4	19	+4	19	+4	22	+4	22	+4	20	+4
8	29	+4	29	+4	30	+4	30	+4	30	+4
12	35	+4	35	+ 4	36	+4	36	+4	36	+4
16	41	+4	41	+4	41	+4	41	+4	40	+4
20	47	+4	47	+4	47	+4	47	+4	45	+4
22	49	+4	49	+4	49	+4	49	+4	47	+4
24	52	+4	52	+4	50	+4	50	+4	50	+4
26	56	+4	56	+4	54	+4	54	+4	53	+4
28	60	+3	60	+4	58	+4	58	+1	57	+4
30	63	+1	63	+2	60	+1	60	+1	60	0
32	65	0	65	+1	62	+4	62	0	62	0
34	67	0	67	+1	64	0	64	0	64	+1
36	69	0	69	+1	66	+1	66	0	66	0
38	71	+1	71	0	67	0	67	0	68	0
40	72	0	72	+1	68	+3	68	0	69	0
43	74	0	74	0	70	0	70	0	70	0
45	75	+1	7 5	+1	71	0	71	0	71	0
50	77	0	77	+1	73	0	73	0	73	0
Final tim	e; 45	mins.	50 m	ins.	40 m	ins.	30 m	ins.	34 m	ins.
Final tem	p.: 75	С	77 C		68 C		60 C		64 C	

Appendix 1. Emulsion internal temperature (EIT) and degree of recovered growth of \underline{S} . schenckii (DRG) at each processing time for each experimental study (continued).

Processing	2	бу	2	7у	28	Ву	29	9m	30m	
time (minutes)	EIT	DRG	EIT	DRG	EIT	DRG	EIT	DRG	EIT	DRG
0	10	+4	10	+4	10	+4	10	+4	10	+4
4	20	+4	20	+4	20	+4	21	+4	21	+4
8	30	+4	28	+4	28	+4	31	+4	31	+4
12	36	+4	34	+4	34	+4	37	+4	37	+4
16	40	+4	40	+4	40	+4	42	+4	42	+4
20	45	+4	44	+4	44	+4	47	+4	47	+4
22	47	+4	47	+4	47	+4	49	+4	49	+4
24	50	+4	49	+4	49	+4	51	+4	51	+4
26	53	+4	54	+4	54	+4	56	+4	56	+4
28	57	+1 .	57	+4	57	+1	60	+4	60	+4
30	60	+1	60	+3	60	0	63	+1	63	+4
32	62	+1	63	+1	63	0	65	0	65	+4
34	64	0	66	+1	66	0	67	0	67	+2
36	66	0	67	0	67	+1	69	0	69	+1
38	68	0	68	0	68	0	71	0	71	+1
40	69	0	70	+1	70	0	72	0	72	+1
43	70	0	72	0	72	0	75	0	75	+1
45	71	0	73	0	73	0	77	0	77	+2
50	73	0	74	0	74	0	79	0	79	0
Final time:	32 m	ins.	40 m	ins.	36 m:	ins.	30 mins.		45 mins.	
Final temp.:	62 C		70 C		67 C		63 C		77 C	

Appendix 1. Emulsion internal temperature (EIT) and degree of recovered growth of \underline{S} . schenckii (DRG) at each processing time for each experimental study.

Processing	3	lm	3	2m	3:	3m	3-	4m	35y		
time (minutes)	EIT	DRG	EIT	DRG	EIT	DRG	EIT	DRG	EIT	DRG	
0	10	+4	10	+4	10	+4	10	+4	10	+4	
4	21	+4	21	+4	22	+4	22	+4	21	+4	
8	32	+4	32	+4	30	+4	30	+4	30	+4	
12	37	+4	37	+4	36	+4	36	+4	36	1 4	
16	41	+4	41	+4	40	+4	40	+4	41	+4	
20	46	+4	46	+4	45	+4	45	+4	48	+4	
22	48	+4	48	+4	48	+4	48	+4	50	+4	
24	50	. +4	50	+4	51	+4	51	+ 4	51	+4	
26	54	+4	54	+4	54	+4	54	+4	55	+4	
28	58	. +4 .	58	+4	57	+4	57	+4	58	+1	
30	61	+2	61	0	61	+4	61	+3	60	0	
32	64	+1	64	+1	63	0	63	0	62	0	
34	67	+1	67	0	65	0	65	+1	65	+1	
36	69	0	69	0	67	0	67	0	67	0	
38	70	0	70	0	69	0	69	0	68	0	
40	71	0	71	0	70	0	70	0	69	0	
43	74	0	74	0	72	0	72	0	70	0	
45	7 5	0	75	0	73	0	73	0	71	0	
50	77	0	77	0	75	0	75	0	73	0	
Final time:	34 m	ins.	32 m	32 mins.		ins.	34 mins.		34 mins.		
Final temp:	67 C		64 C		61 C		65 C	18 327	65 C		

Appendix 1. Emulsion internal temperature (EIT) and degree of recovered growth of \underline{S} . schenckii (DRG) at each processing time for each experimental study (continued).

Proces		3	бу	3.	37у		38y		3 ⁹ y		40y	
tim		EIT	DRG	EIT	DRG	EIT	DRG	EIT	DRG	EIT	DRG	
0		10	+4	10	+4	10	+4	10	+4	10	+4	
4		21	+4	20	44	20	+4	20	+4	20	+4	
8		30	+4	30	+4	30	+4	30	+4	30	+4	
12		36	+4	36	+4	36	+4	36	+4	36	+4	
16		41	+4	41	+4	41	+4	41	+4	41	+4	
20		48	+4	46	+4	46	+4	48	+4	48	+4	
22		50	+4	49	+4	49	+4	50	+4	50	+4	
24		51	. +4	52	+4	52	+4	52	+4	52	+4	
26		55	+1	56	+ 4	56	+4	55	+4	55	+2	
28		58	. 0 .	57	+4	57	+1	60	+3	60	0	
30		60	0	60	+2	60	+1	64	0	64	0	
32		62	+2	62	+4	62	+1	66	+1	66	0	
34		65	41	63	+1	63	+1	67	0	67	0	
36		67	0	65	+1	65	+2	68	0	68	0	
38	¥	68	0	66	+1	66	0	69	0	69	0	
40		69	0	68	+1	68	+4	70	0	70	0	
43		70	0	70	0	70	0	72	0	72	0	
45		71	0	71	+1	71	+2	73	0	73	0	
50		73	0	74	0	74	+1	7 5	0	75	0	
Final	time:	34 m	ins.	45 m	ins.	50 m:	ins.	32 m	ins.	26 m	ins.	
Final	temp.:	65 C		71 C		74 C		66 C		55 C		

Appendix 1. Emulsion internal temperature (EIT) and degree of recovered griwth of \underline{S} , schenckii (DRG) at each processing time for each experimental study (continued).

Proces		4	1m	4:	42m		3m	4	4m	45m		
tim		EIT	DRG	EIT	DRG	EIT	DRG	EIT	DRG	EIT	DRG	
0		10	+4	10	+4	10	+4	10	+4	10	+4	
4		20	+4	20	+4	20	+4	20	+4	20	+4	
8		30	+4	30	+4	30	+4	30	+4	29	+4	
12		35	+4	35	+4	36	+4	36	+4	35	+4	
16		40	+4	40	+4	40	+4	40	+4	39	+4	
20		44	+4	44	+4	44	+4	44	+4	43	+4	
22		46	+4	46	+4	46	+4	46	+4	46	+4	
24		49	+4	49	+4	50	+4	50	+4	49	+4	
26		52	+4	52	+4	52	+4	52	+4	52	+4	
28		56	+4	56	+4	56	+4	56	+3	55	+4	
30		60	+4	60	+4	60	+4	60	0	60	1 4	
32		62	+1	62	+4	62	+3	62	+1	62	+1	
34		65	0	65	+3	64	0	64	+1	64	+1	
36		68	0	68	+1	65	0	65	0	66	+1	
38		70	0	7 0	+1	67	0	67	+3	67	+1	
40		71	0	71	+1	69	0	69	+1	69	0	
43		73	+1	73	+1	71	0	71	0	71	+1	
45		75	0	75	+1	72	0	72	0	72	0	
50		76	0	76	+1	74	0	74	0	74	0	
Final	time:	43 m	ins.	50 mi	50 mins.		32 mins.		40 mins.		43 mins.	
Final	temp.:	73 C		76 C		62 C		69 C		71 C		

Appendix 1. Emulsion internal temperature (EIT) and degree of recovered growth of \underline{S} . schenckii (DRG) at each processing time for each experimental study (continued).

Processing time		4	6m	47y		48	48y		49y		Э у
tim (minut		EIT	DRG	EIT	DRG	EIT	DRG	EIT	DRG	EIT	DRG
0		10	+4	10	+4	10	+4	10	+4	10	+4
4		20	+4	20	+4	20	+4	20	+4	20	+4
8		29	+4	28	+4	28	+4	30	+4	30	+4
12		35	+4	34	+4	34	+4	36	+4	36	+4
16		39	+4	40	+4	40	+4	42	+ 4	42	+4
20		43	+4	44	+4	44	+4	46	+4	46	+4
22		46	+4	46	+4	46	+4	48	+4	48	+4
24		49	. +4	49	+4	49	+4	51	+4	51	+4
26		52	+4	51	+4	51	+4	54	+4	54	+3
28		55	0 .	56	+1	56	0	56	+4	56	+1
30		60	0	59	+1	59	0	60	+1	60	0
32		62	+1	61	+1	61	0	62	0	62	0
34		64	0	64	+1	64	0	64	0	64	0
36		66	0	66	0	66	0	66	0	66	0
38		67	0	68	0	68	0	67	0	67	0
40		69	0	70	0	70	0	68	0	68	0
43		71	0	72	0	72	0	71	0	71	0
45		72	0	73	0	73	0	72	0	72	0
50		74	0	74	0	74	0	74	0	74	0
Final	time:	32 m	ins.	34 m	ins.	26 m:	ins.	30 m	ins.	28 m	ins.
Final	temp.:	62 C		64 C	4400	51 C		60 C		56 C	

Appendix 1. Emulsion internal temperature (EIT) and degree of recovered growth of \underline{S} , schenckii (DRG) at each processing time for each experimental study (continued).

Proces		51m		5:	52m		53m		54m		55y	
tin (minut		EIT	DRG	EIT	DRG	EIT	DRG	EIT	DRG	EIT	DRG	
0		10	#4	10	+4	10	+4	10	+4	10	+4	
4		19	+4	19	+4	22	+4	22	+4	19	+4	
8		30	+4	30	+4	31	+4	31	+4	29	+4	
12		36	+4	36	+4	37	+4	37	+4	35	+4	
16		40	+4	40	+4	41	+4	41	+4	40	+ 4	
20		45	+4	45	+4	47	+4	47	+4	46	+4	
22		47	+4	47	+4	49	+ 4	49	+4	48	+4	
24		50	+ 4	50	+4	52	+4	52	+4	51	+4	
26		53	+4	53	+4	55	+4	55	+4	56	+4	
28		57	. +4	57	+4	59	+4	59	+1	59	+2	
30		60	+4	60	+1	61	+1	61	+1	61	0	
32		63	0	63	+2	64	0	64	0	63	0	
34		65	0	65	0	66	0	66	0	65	0	
36		67	0	67	0	68	0	68	0	68	0	
38		69	0	69	0	70	0	70	0	69	0	
40		71	0	71	0	71	0	71	0	70	0	
43		73	0	73	0	73	0	73	0	72	0	
45		74	0	74	0	75	0	75	0	73	0	
50		76	0	76	0	76	0	76	0	75	0	
Final	time:	30 m	ins.	32 m:	ins.	30 m	ins.	30 m	ins.	28 m	ins.	
Final	temp.:	60 C	9900	63 C		61 C		61 C		59 C		

Appendix 1. Emulsion internal temperature (EIT) and degree of recovered growth of <u>S. schenckii</u> (DRG) at each processing time for each experimental study (continued).

Processing	5	56y		57y		58y		59y		60y	
time (minutes)	EIT	DRG	EIT	DRG	EIT	DRG	EIT	DRG	EIT	DRG	
0	10	+4	10	+4	10	+4	10	+4	10	+4	
4	19	+4	21	+4	21	+4	19	+4	19	+4	
8	29	+4	30	+4	30	+4	29	+4	29	+4	
12	35	+4	37	+4	37	+4	35	+4	35	+4	
16	40	+4	42	+4	42	+4	42	1 4	42	+4	
20	46	+4	49	+4	49	+4	48	+4	48	+4	
22	48	+4	51	+4	51	+4	50	+4	50	+4	
24	51	+4	54	+4	54	+4	53	+4	53	+4	
26	56	+4	58	+4	58	+ 4	57	+4	57	+1	
28	59	О.	61	+4	61	+2	61	+4	61	+1	
30	61	+2	63	+1	63	+2	64	+1	64	+1	
32	63	0	66	0	66	+1	67	0	67	0	
34	65	0	68	0	68	+1	70	0	70	0	
36	68	+1	69	0	69	+1	73	0	73	0	
38	69	0	70	0	70	+1	74	0	74	+1	
40	70	0	71	0	71	+1	75	0	75	0	
43	72	0	72	0	72	0	76	0	76	0	
45	73	0	73	0	73	+1	7 7	0	77	0	
50	75	0	7 5	0	75	0	80	0	80	0	
Final time:	36 m	ins.	30 m	ins.	45 mins.		30 mins.		38 mins.		
Final temp.:	68 C		63 C	E - MANUAL MANAGER	73 C		64 C		74 C		

Appendix 1. Emulsion internal temperature (EIT) and degree of recovered growth of \underline{S} . schenckii (DRG) at each processing time for each experimental study (continued).

	Emulsion	internal t	emperature d	Rounded data		
Processing time (minutes)		Standard deviation (s)	Variance (s2)	Standard error of mean	(<u>x</u>)	(s)
0	10.00	0.0000	0.0000	0.0000	10	0
4	20.42	1.0048	1.0097	0.1297	20	1
8	29.90	1.0116	1.0233	0.1306	30	1
12	35.85	1.0376	1.2942	0.1469	36	1
16	40.85	1.1807	1.3942	0.1524	41	1
20	46.12	1.6640	2.7030	0.2123	46	2
22	48.42	1.6960	2.8760	0.2190	48	2
24	51.00	1.5383	2.3667	0.1936	51	2
26	54.35	1.8423	3.3942	0.2378	54	2
28	58.17	1.8544	3.4389	0.2394	58	2
30	61.18	1.6072	2.5831	0.2075	61	2
32	63.53	1.7075	2.9156	0.2204	64	2
34	65.70	1.8009	3.2433	0.2325	66	2
36	67.65	1.8870	3.5608	0.2436	68	2
38	69.08	1.8008	3.2431	0.2325	69	2
40	70.43	1.6770	2.8122	0.2165	70	2
43	72.22	1.7615	3.1031	0.2274	72	2
45	73.33	1.8227	3.3222	0.2353	73	2
50	75.22	1.7990	3.2364	0.2322	75	2
	(X)	(s)	(s ²)	(S.E. X)	(<u>x</u>)	(s)
Final time:	37.47 mins	. 6.9149 m	ins. 47.8156	0.8927	37mir	ns. 7mins.
Final temp.:	67.47 C	6.0923 C	37.1156	0.7865	67 C	6 C

SURVIVAL OF SPOROTHRIX SCHENCKII IN A MEAT PRODUCT OF ANIMAL ORIGIN

by

JOHN HOWARD SCHARDING
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AN ABSTRACT OF A MASTER'S THESIS

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Department of Infectious Diseases

KANSAS STATE UNIVERSITY Manhattan, Kansas

In 1969, it was reported for the first time that the pathogenic fungus Sporothrix schenckii had been found in commercially processed frankfurters. Except for this single report, there are few references in the literature concerning the ability of this organism to survive the processing procedures that frankfurters or other meat products undergo. Since such a capability could have an impact on the meat industry and public health agencies, experimental studies were undertaken to determine to what extent S. schenckii can survive these processing procedures.

A suitable frankfurter test model was devised using a polypropylene test tube which had the walls of the lower portion of the tube partially removed, and a section of cellulose casing affixed to the exterior of the model. Raw frankfurter emulsion inoculated with <u>S. schenckii</u> was stuffed into the model and compressed with a plunger. A similar model was also filled with the emulsion and a thermometer inserted for recording the internal temperature. Both models were placed in a laboratory oven and cooked for 24 minutes at an oven temperature of 85 C, plus 26 minutes at 100 C. Recovery of the organism was attempted approximately every two minutes over the 50 minutes of experimental processing time.

The data shows that the frankfurter test model and methods adequately simulate the conditions employed in the processing of most commercial frankfurters. Results of 60 experimental studies indicate that the mean of the final processing times for recovery of the organism was 37 ± 7 minutes, with a range of 26 to more than 50 minutes. The mean

of the corresponding final internal temperatures was 67 ± 6 C, with a range of 51 to more than 77 C. Few commercial producers process their frankfurters more than 43 minutes, or to a higher final internal temperature than 71 C. The final recovery times and internal temperatures in 32 % of the experimental studies met or exceeded these standards.

Thus, it is possible that <u>S. schenckii</u> can survive the processing procedures used in the manufacture of many commercial frankfurters, and could be a potential health hazard.