Vicente Da Cruz at al., there are pages missing.

The aim of the present work is to clarify some of these questions, specially those concerned with the relationship between frosting and defrosting conditions and the *S. aureus* development and enterotoxin production. It also concerns the effect of cooking on the destruction of *S. aureus* and the enterotoxin.

MATERIALS AND METHODS

1 - Samples

6 samples of fresh turkey meat were carried from the slaughterhouse to the laboratory at + 4°C, and mantained at this temperature until the next morning for examination.

2 - Cell suspension preparation

- One strain of *Staphylococcus aureus* producer of Enterotoxin A was obtained from LNETI-DTIA Culture Collection.

- The strain was grown in Tryptone Soya Broth (OXOID) at 37°C for 24h; Cells were centrifuged at 5000 r.p.m. for 10 mins.; Washed twice with saline (0,85% Na Cl); Resuspended in saline and adjusted to about 10^6 cells/ml using a photometer (Ultrospec II - LKB) at 600 nm.

3 - Samples preparation

- Each sample was split into 13 portions of 20g.

- 1 Portion (A) was immediately examined for enumerationof *S. aureus* and quantification of enterotoxin.

- 12 Portions (B to M) were inoculated with the cell suspension of *S. aureus* (1 ml).

- B, C, D and E portions were incubated for 24 h at 25°C

- F, G, H and I portions were incubated for 48 h at 25°C.

- J, K, L and M portions were incubated for 72 h at 25°C.

- B, F and J portions were examined after the incubation period for the enumeration of *S. aureus* and quantification of enterotoxin.

- All the other portions were frosted at -18°C for 12 days.

- C, G and K portions were defrosted at + 7°C for 18 h.

- D, H and L portions were defrosted at + 20°C for 2 h.

- E, I and M portions were boiled for 15 mins.

4 - Enumeration of S. aureus

The number of *S. aureus* was determined on Baird Parker Agar (OXOID) using a surface drop method. Colonies were confirmed as *S. aureus* by coagulase production (ISO 6888).

5 - Enterotoxin extraction procedure

- Homogenization of all the portions (72) with 20 ml of PBS in a Stomacher for about 4 mins.

- Mantained at 37°C for 60 mins.

- Centrifugation at 900 g at 4°C for 30 mins.

- Filtration of the supernatant through Whatman paper nº 42 and 40.

- Filtration of the liquid through membrane filter (Millipore GVWPO2500) and retaining the filtrate to determine the toxin content.

6 - RPLA Test

- Extracts were tested according to the manufacturer's (OXOID) instructions, using control and SE sensitized latexes.

- Results were scored as either negative (bottom), positive (agglutination) or non-specific reaction (NSR) after incubation for 18 - 20 h at 20°C.

- To determine the smallest amount of toxin that could be detected in foods 0,002 µg of SEA was added to 200 µl of extract obtained from A portion of each sample.

RESULTS

- All the samples examined without artificial contamination didn't reveal the presence of *S. aureus* nor the presence of enterotoxin A. The volume of extract obtained is 15,9 ml/20 g.

- The minimum detectable limit of Staphylococcal enterotoxin A (sensitivity) in turkey meat was 2,32 ng/g.

- The enumeration of *S. aureus*, the volume of extracts obtained and the quantification of enterotoxin A produced are presented in tables 1 to 3 (24 h, 48 h and 72 h).

TABLE 1

Staphylococcus aureus (log cfu/g average) and Enterotoxin A (average) in artificially contaminated turkey meat

Incubation - 24h at 25°C

Portions		S. aureus log cfu/g	Volume of extract ml/20g	Enterotoxin A ng/g
No frosted		7,61	14,7	13,34
Defrosted	20°C - 2h	7,76	14,8	13,92
	7°C - 18h	7,39	15,4	9,28
Boiled		<1,00	16,1	No detectable

TABLE 2

Staphylococcus aureus (log cfu/g average) and Enterotoxin A (average) in artificially contaminated turkey meat

Incubation - 48 h at 25°C

Portions		S. aureus log cfu/g	Volume of extract ml/20g	Enterotoxin A ng/g
No frosted		7,42	13,7	5,42
Defrosted	20°C - 2h	7,41	12,9	5,42
	7°C - 18h	7,11	12,6	3,71
Boiled		<1,00	14,8	No detectable

TABLE 3

Staphylococcus aureus (log cfu/g average) and Enterotoxin A (average) in artificially contaminated turkey meat

Incubation - 72 h at 25°C

Portions		S. aureus log cfu/g	Volume of extract ml/20g	Enterotoxin A ng/g
No frosted		7,55	12,9	3,48
Defrosted	20°C - 2h	6,66	10,6	6,96
	7°C - 18h	6,58	10,4	3,48
Boiled		<1,00	16,2	No detectable

CONCLUSIONS:

- The enumeration of *Staphylococcus aureus* is maximum at 24 hours and maintained during the incubation periods of 48 and 72 hours; the slow defrosting revealed lower counts than the rapid one; the populations obtained after 24 hours of incubation allowed the formation of detectable amounts of Enterotoxin; the boiling step destructed allways the viable cells of *Staphylococcus aureus*.

- The volume of meat extract obtained vary according to the treatment of the sample. So, the volume is bigger for the uncontaminated samples, and smaller for the inoculated ones, decreasing gradually from the 24 hours incubation to the 72 hours incubation. The type of defrosting don't change significantly the volume of the extract obtained. The boiling of the meat causes the obtention of greater volumes.

- The detectable amount of Enterotoxin decreases during the incubation time and it is always smaller with slow defrosting (18 h at 7°C) than with the rapid one (2 h at 20°C). There is no detectable amount of Enterotoxin in any of the boiled samples.

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