EVALUATION OF MINIMUM THERMAL TREATMENT REQUIRED FOR THE DESTRUCTION OF *L.MONOCYTOGENES, E.COLI* O157:H7 AND *SALOMNELLA* SPP. FOR FRANKFURTER AND PARISA PROCESSING.

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Introduction

L.monocytogenes, E.coli O157:H7 and *Salmonella* spp, are human pathogens of paramount importance for meat industry (Padye and Doyle, 1991, 1992; Pinner et al, 1992; Mermelstein, 1993). Survival of the pathogens during cooking has been a contributing factor in most outbreaks (Schwartz et al, 1988; Anonymous, 1991; McDonough et al, 1991; Mermelstein, 1993).

Commercial thermal processing for cured cooked meat products varies depending on the kind of product and the manufacturer thus the evaluation of its efficacy is difficult. FP-value has been established as a value that helps to estimate the thermal processing no matter which is the maximum core temperature, its fluctuations and the processing time. FP_{70} value is the analogous of $F_{121,1}$-value for sterilisation with reference temperature 70^0C and Z value 10^0C, and thus it represents the time in minutes at 70^0C that has the same result with any other combination of temperature and time.The evaluation of the necessary minimum FP_{70}-value for the destruction of these pathogens in cured cooked meat products offers important data to pasteurized meat product technology defining the lower margin of their thermal treatment.

Materials and methods

Strains and preparation of inoculum

As test micro-organisms we used *L.monocytogenes* strains Scott-A, V7, VPH1, VPH4 and RM, three strains of *E.coli* O157:H7 and *Salmonella enteritidis, S.typhimurium, S.infantis, S.marina, S.havana* and *S.seftenberg* 775W.

Stock cultures were kept on BHI agar slants (OXOID) at 4^0C. Specific bacterial inocula were prepared by two consecutive subcultures in tubes containing BHI broth (OXOID) incubated at 37^0C for 24 hours. Pools of strains of the same species were prepared by mixing 1ml from each individual strain culture. The tubes were centrifuged at 3.000 rpm for 10 min and the cells were resuspended in 0,1% (w/v) peptone water. After a second centrifugation the bacterial cells were resupended in peptone water 0,1% so that the initial cfu/ml was about 2-4 X 10^9.

Experimental design

Raw sausage mixture for frankfurters and parisa, obtained from a local plant, were transferred to the laboratory into an isothermic box at 0^0C, within one hour of preparation.

Frankfurter formulation contained porc meat 30%, fat 16%, collagen 12%, starch 5%, milk serum 2%, dextrose 1%, salt 1,8%, nitrite 0,02%, nitrate 0,015%, phosphates 0,25% and seasoning 1,5%.

Parisa contained porc meat 33%, total fat 17%, collagen 10%, starch 5%, milk serum 2%, lactose 0,5%, dextrose 0,25%, salt 1,5%, nitrite 0,012%, nitrate 0,008%, phosphates 0,25% and seasoning 1,2%.

From each formulation 1 g portions were stuffed into finger cots which were next injected with 0,01 ml of each bacterial inoculum to get 2-4 X 10^7 cells/finger cot. The entrapped air was evacuated with a syringe, a knot was tied and the finger cot was placed into the geometric center of a blood collecting tube, 14 mm diameter, which was filled with the same raw sausage mixture. The tubes, containing about 10 g of sausage, were closed with a rubber cap. A tip of the thermocouple and a needle, to leave the air to escape during thermal treatment, were pierced through the rubber cap into the geometric center of the tube. Eight tubes containing the strains of one pathogen were thermally treated in a water bath at $70\pm0,2\ ^0$C.

FP-value was monitored by a thermocouple (model CTF 9008, ELLAB, Copenhagen, Denmark). At predetermined FP values a tube was picked up from the water bath and placed into slashed ice. The calculation of FP value continued during cooling until no further growth of the value to be noticed.

Detection of survivors

After cooling the finger cots were taken out of the tubes aseptically, were cut to small pieces and placed into sterile screw cap tubes where they were pulverised further with a flat end glass rod. Enrichment broth was added according to each pathogens individual procedure, and the tubes were vortexing for two minutes.

Detection of *L.monocytogenes.* 9 ml of FDA Listeria enrichment broth (LEB) without antimicrobials, was poured into the tubes containing the pulverised samples. The tubes were incubated at 30^0 C/24h (first enrichment step). Next day 0,1 ml from all tubes were transferred into tubes containing 9 ml LEB with antimicrobials (nalidixic acid 40 mg/Lt, cycloheximide 15 mg/Lt and acriflavine 50 mg/Lt) and tubes containing 9 ml of Frazer broth (BBL). All tubes were incubated at 30^0 C/24h (second enrichment step).After incubation a loopful from each tube was streaked onto LPM agar (BBL) which was incubated at 37^0 C/24-48 h. Three suspected colonies were transferred onto BHI agar (OXOID) to ensure purity and confirmed as *L.monocytogenes* by Gram staining, testing for motility, catalase production, utilisation of esculin, rhamnose, mannitol, xylose and a-methyl-d-mannopyranoside, production of beta-hemolysin on sheep blood agar and Camp-test (Genigeorgis et al, 1990; Cowan, 1986).

Detection of *Salmonella* spp. Buffered Peptone Water was used for primary enrichement. After incubation at 37^0C for 24 hours, 0,1 ml was transfered from each tube to Selenite-Cystine broth (OXOID). They were icubated at 37^0C for 24 hours (second enrichement). A loopful from each tube was then streaked onto XLD agar (BBL) and SS agar (OXOID). The plates

were incubated at 37^{0}C for 24-48 hours. Presumptive *Salnonella* isolates were then biochemically tested on TSI agar (OXOID) and LI agar (OXOID) together with urease and indole tests.

Detection of *E.coli* O157:H7. 9 ml of TSYE broth (BBL) was added in each tube containing the pulverized samples. The tubes were left for 1 hour at room temperature. Then 1 ml from each tube was streaked onto MacConkey sorbitol agar (MSA, BBL) which was incubated at 37^{0}C for 17 to 24 hours. Sorbitol negative colonies were considered as *E.coli* O157:H7.

Results and Discussion

In this study FP_{70}values of 1,95; 2,54 min for Pariza and 2,28 and 2,80 min for frankfurter were estimated for 7,2 DR of *L.monocytogenes* and *Salmonella* spp respectively. FP_{70} of 1,29 for pariza and 1,76 min for frankfurter resulted in 6,3 DR of *E.coli* O157:H7. These results are in accordance with the literature.

The reported D_{70}values concerning *L.monocytogenes* are 0,23 min in meat slurry (Boyle et al,1990); 0,14 in raw beef, 0,11 in chicken leg and 0,13 min in chicken breast (Mackey and Bratchell,1990). Gaze et al., 1989, recomend that the slowest heating point in a product should be held at 70^{0}C for 2 min to be effectively decontaminated of *L.monocytogenes* (6 log reduction). Reportedly *E.coli* O157:H7 has shown no unusual heat resistance with D-values at 64,3^{0}C in ground beef, 9,6sec (Doyle and Schoeni,1984) and at 62,8^{0}C in ground beef with 30% fat, 0,47 min (Line et al.,1991). Ahmed et al.,1995, reported that D_{60}-values for *E.coli* O157:H7 ranged from 0,45-0,47 in beef, 0,37-0,55 in pork sausage (7-30% fat), 038-0,55 in chicken and 0,55-0,58 in turkey. D_{68} of 0,12 min have been reported in ground beef (Orta-Ramirez et al.,1997) and D_{60} of 2,4 min in frankfurter with 17% fat (Kotrola et al., 1997). Fat content affected lethality resulting in higher D-values.Ahmed et al., 1995, suggest that providing the product an internal temperature of 60^{0}C for 2-3 min would result a ≥5D reduction of *E.coli* O157:H7. Goodfellow and Brown, 1978, reported that $D_{62,7}$-value for *Salmonella* spp in roast beef was 0,6-0,7 min and Orta-Ramirez et al., 1997, reported D_{68} 0,22 min in ground beef for *S.senftenberg* .

Sergelidis et al.,1995, reported that the estimated FP_{70}-values, during commercial thermal treatment in Greece, ranged 23-373,5 min for frankfurters and 34-78,5 min for Pariza. Although these processes seem to be exessive in order to achieve food safety it is suggested (Bogh-Sorensen,1994) that FP_{70}-values for pasteurized meat products should be >40 min to attain an adequate shelf life. For large products thermal treatment lasts many hours in order to achieve an adequate core temperature (71-76^{0}C) which sometimes results in FP-values around 500 (Bogh-Sorensen,1994).

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