

SESSION 2. MICROBIOLOGY AND HYGIENE

2:1

The relation between water activity and microbial spoilage in meat product models

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The aim of the study was to determine the influence on bacterial growth of water activities (a_w) caused by different chloride salts. A few experiments were done using NaCl, KCl and $MgCl_2$ in broth cultures at a_w values resembling those obtained with sodium chloride only in cured, cooked meat products such as Bologna sausage (with an approximate brine concentration of 4.0). Further, the influence of type of salt on the minimum a_w allowing growth was examined. Finally, a study was done on the effect of increasing a_w accomplished by sodium chloride in a meat product.

Microbial development at different temperatures was followed in broth cultures with NaCl, KCl and $MgCl_2$ by O.D. measurements using a spectrophotometer. The study of sliced, vacuum-packed Bologna sausage was done on three batches of sausage produced with different NaCl concentrations. The sausages were sliced and vacuum-packed under commercial conditions. Appropriate dilutions of meat sausage homogenate in peptone water were plated on agar media. The sausages were also evaluated organoleptically by a taste panel.

The O.D. measurements showed that $MgCl_2$ was a stronger inhibitor than NaCl and KCl and that an effect of KCl compared to NaCl, if present, was more pronounced at otherwise inhibitory conditions. Further, that the minimum a_w at optimal growth temperature for the organisms varied with type of salt for some bacteria. Thus, $a_{w, min}$ for growth with NaCl and KCl were often equal, but it was higher with divalent metal chlorides. The meat sausage study which was

done only on NaCl, showed that at increasing a_w (decreasing salt concentration) the proportion of Micrococaceae fell and that of Brochothrix thermosphacta and the Gram negative bacteria increased. The effect on the lactics was primarily an increased lag phase especially at low temperatures. This resulted in a longer shelf life at increased a_w especially at slightly elevated storage temperature.

In conclusion, especially $MgCl_2$ and $CaCl_2$ strongly inhibited bacterial growth, but previous studies have shown that these salts are not organoleptically acceptable. Though KCl inhibits at low a_w to the same extent as NaCl, high concentrations of KCl are not organoleptically acceptable. When using high salt concentrations in controlling bacterial growth one therefore inevitably ends up with NaCl. This salt was shown to increase the storage period for vacuum-packed sliced Bologna, without making the product unfit for consumption. The effect was observed especially at slightly elevated temperature (10°C).

2:2

DEGRADATION OF BOVINE LEAN MEAT UNDER THE CHILLED STORAGE CONDITION

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SUMMARY

The effects of *Pseudomonas fragi* and *Proteus vulgaris* on deep, block bovine lean meat samples after 0, 3, 7 and 14 days of storage in comparison with uninoculated control samples were studied in five replicate samplings. Results of the microbial analysis had shown more rapid growth of *Ps. fragi* than *Pr. vulgaris* on meat samples while minimal counts were enumerated on the control samples at the end of storage time. Total volatile basic nitrogen and amino nitrogen quantities of the inoculated muscle samples increased during storage with *Ps. fragi* exhibiting greater proteolytic activity than *Pr. vulgaris*. A slight increase was noted for the uninoculated control samples at the end of storage.

Scanning electron microscopy results have shown the muscle fibers from *Ps. fragi*-inoculated samples exhibited more breakages

than *Pr. vulgaris*-inoculated samples as a probable result of spoilage. On the myofibrillar level, results have shown that no major proteolysis occurred, thus supporting the results of the previous protein analysis by SDS gel electrophoresis.

INTRODUCTION

Rapid deterioration of foods is mainly the consequence of bacterial action, specifically in meat whose moisture content with its rich load of nutrients in solution provides a favorable medium for bacteria. Just as *Ps. fragi* is a proteolytic bacteria (HASEGAWA et al., 1970; DUTSON et al., 1971; TARRANT et al., 1973), *Pr. vulgaris* is also known to produce a strong putrefactive odor in a protein-containing media and for that reason it was selected in this study.

MATERIALS AND METHODS

Bovine muscle blocks were washed with 70% EtOH to eliminate surface bacterial contamination. To the prepared inoculum, muscle blocks were carefully dipped in tanks for about 30 seconds and then layed in a plastic container and stored at 2°C. Stored muscle blocks were determined by bacterial counts, volatile basic nitrogen, amino nitrogen, SDS-PAGE patterns and SEM observation.

RESULTS

Loosening of the myofibrils is displayed in a result of the formation of intermyofibrillar spaces. No disruption of the I and A bands, and the Z line were evidently observed except for a slight damage observed within the I band region. This was rather believed to be an evidence of the removal of materials from the Z lines as caused by microbial growth.

2:3

MICROBIOLOGICAL PROBLEMS OF MECHANICALLY DEBONED PORK MEAT PRODUCTION AND PROCESSING

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Mechanical deboning is a process which separates meat and some bone marrow from bones. Mechanically deboned meat (MDM) is an ideal medium for microbial growth because it is very finely minced. Hence, further studies are needed to determine whether the use of MDM poses a health hazard.

The purpose of this study was to evaluate the microbiological quality of mechanically deboned pork meat obtained from a Seffelaar-Looyen deboner. The microbiological state was determined in the meat from bones before the process of deboning and in mechanically deboned meat just after deboning. MDM was salted (2% NaCl) and cured with different doses of NaNO₂. MDM without any additions, after salting and after curing was stored at the temperature 2-4°C for 1, 2, 3, 4 and 6 days, and at -18°C for 4 and 8 weeks. Also hamburgers with the a 15% addition of MDM, were prepared. Hamburgers were stored at -18°C for 4 and 8 weeks. Microbiological analysis were done before and after heat treatment /80°C for 10 minutes/. Aerobic plate counts, coliforms and enterococci were determined in all samples.

2:4

THE USE OF AUTOTRAK FOR THE RAPID ENUMERATION OF MICRO-ORGANISMS ON MEAT SURFACES

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SUMMARY

The ability of AUTOTRAK to count the number of micro-organisms on the surfaces of meat carcasses was compared with that of the total viable count (TVC) obtained using the standard pour plate method. A regression value of $r=0.82$ was obtained from the 110 samples examined.

It was concluded that AUTOTRAK is a successful method for the rapid non-destructive assessment of the microbial quality of meat surfaces.

INTRODUCTION

Destructive methods of meat sampling, such as maceration, result in the devaluation of the meat carcass. Methods which cause minimal damage are more acceptable to the meat suppliers thus, surface rinsing or swabbing are commonly used for meat sampling. AUTOTRAK, a rapid automated system for the enumeration of micro-organisms in liquid menstrua developed by the Centre for

It was found that the process of mechanical removing of meat from bones influenced deterioration of the microbiological quality of the obtained MDM. A steady increase in the number of microorganisms took place during storage under refrigeration.

NaCl restrained the development of *E. coli*, but it did not influence the development of enterococci. Curing with the addition of nitrite not lower than 0.01% in relation to the weight of the sample, caused a partial inhibition of bacterial growth in comparison to that in the MDM without any preservatives and with the addition of a smaller dose of nitrite.

Freezing and storage in the frozen state of cured and not cured MDM, caused a nearly 10-fold decrease of the total number of aerobic microorganisms.

An addition of deboned meat to hamburgers increased their contamination. Heating of hamburgers to 80°C, improved their microbiological state.

It was concluded that MDM should not be stored under refrigeration, but can be used just after obtaining in cooked products

Bio-medical Instrumentation in the University of Strathclyde, is based on the principle of Direct Fluorescence Microscopy. Sample is applied to a moving tape, fluorescently stained, and passed under a microscope. The signals generated are amplified by a photomultiplier and digitised to produce a readout expressed as the number of micro-organisms per millilitre.

The results obtained from AUTOTRAK were compared with those obtained from the standard colony count procedure.

MATERIALS AND METHODS

The skin surface of beef flank samples were swabbed using a template of known area (4cm²). The swab was transferred to 10ml of 1/4 strength Ringers solution and mixed for 1 minute at ambient temperature. A 2ml aliquot was pipetted into a sample cup and presented to AUTOTRAK.

Serial dilutions of each sample in Ringers solution were prepared to cover a range from 0 to 10⁶ organisms per ml. Pour plates were prepared using Plate Count Agar and incubated at 30°C for 3 days.

RESULTS AND DISCUSSION

The results obtained from both the cultural method and AUTOTRAK were plotted and the linear regression calculated. The equation of the line was $\hat{Y} = 1.63 + 0.79X$ and $r=0.82$ for 110 samples. The results were then expressed as micro-organisms per cm².

2:5

COMPARISON OF THE METHOD OF DEFROST OF PRE-COOKED FROZEN PRODUCTS FOR MICROBIOLOGICAL ANALYSIS

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The first problem the Lab has to face when we attempt to analyse a sample of frozen food is that of its preparation, which begins almost inevitably by its defrost.

Now we have to face two alternatives: either a quick defrost at a relatively high temperature (45°C) for a short period (30 min) or a slow one at a fairly lower temperature (5°C) for a frankly longer time (18h).

In order to choose the more correct option we have tested in this work comparatively, these two methods of defrost, considering the frozen sample as the pattern and determining the enumeration of aerobic mesophilic microorganisms, the enumeration of psychrotrophic microorganisms and the enumeration of yeasts and molds.

The analysis concerned a total amount of 20 samples formed by wraps of small pastry or pies which had previously been baked, then frozen and then re-baked before consuming. The techniques used were the following: for the enumeration of aerobic mesophilic microorganisms, the ISO 2293, for the enumeration of psychrotrophic microorganisms, the ISO/DIS 6730, and for the yeasts and molds the counting was made by means of Cooke Rose Bengal + Chlorotetracycline spreaded on surface according to the Portuguese Standard NP 3277/1.

The results we have found allow us to conclude that the method of slow defrost is the one that presents enumerations more different than those found for the sample analysed without defrost, while the method of quick defrost allows us to obtain results clearly much nearer to those of the frozen sample, in relation to any of the parameters under research. It is still remarkable that it is the enumeration of mesophilic microorganisms the one that suffers a greater alteration between the value obtained for the sample without defrost and the one that defreezes slowly.

2:6

PREVALENCE OF ACID-FAST BACILLI IN THE MUSCLE TISSUE OF SLAUGHTER PIGS WITH TUBERCULOUS-LIKE LESIONS IN THE LYMPH NODES

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The objective of the study was to clear up the etiology of tuberculous-like lesions in the submandibular and mesenteric lymph nodes of slaughter pigs and to determine if the same causative agent was disseminated in their muscle tissue. Pathologically changed sections of the lymph nodes and samples of the masseter, sternomandibularis, biceps brachii, gracilis muscles were collected and microbiologically examined. Isolated mycobacteria were subjected to cultural, biochemical and serological examinations. Altogether 287 animals with pathologically changed lymph nodes were examined. 199 strains of *Myc. avium* and 42 atypical bacilli from the lymph nodes of 230 /80.1% slaughter pigs were isolated.

Myc. tuberculosis and *Myc. bovis* were not found. Of 861 samples of muscles 22 /all from 16 animals/ were infected with acid-fast bacilli. 23 strains were isolated, from which 15 belonged to *Myc. avium* and 8 were atypical bacilli. In 14 cases the strains isolated from muscles had some different serological or biochemical properties than those from the lymph nodes. No relationship was found between prevalence of mycobacteria in the lymph nodes and muscles of slaughter pigs.

2:7

MICROBIAL SPOILAGE AND SUBSTRATE CONCENTRATION OF NORMAL AND DFD BEEF

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At 24 h. post mortem samples (\pm 1kg) of the LD-muscles adjacent to the 8th-10th vertebra were cut from 3 DFD beef carcasses ($\text{pH}_{24\text{LD}} = 6.56$) as well as from 3 normal beef carcasses ($\text{pH}_{24\text{LD}} = 5.63$).

At the laboratory the samples were ground and wrapped in polyethylene foël with high oxygen permeability.

Following aerobic storage ($+2^\circ\text{C}$) for 2, 8, 13 and 16 days respectively portions of the samples were examined for total aerobic count (22°C), pseudomonas, Brochotrix thermosphacta and enterobacteriaceae.

In addition glucose, lactic acid and total volatile N-compounds were determined; eventually an evaluation of meat flavour and appearance was made.

The initial contamination (2 days p.m.) with psychrotrophic bacteria for both normal and DFD meat samples was low and amounted to 3.53 and 3.77 respectively ($\log\text{N}/\text{gram}$).

During the entire storage period (16 days) the mean counts of psychrotrophic organisms, pseudomonas and Brochotrix thermosphacta in the DFD ground beef were higher as compared with normal beef.

The initial amount of glucose and lactic acid for the LD samples presenting normal pH_{24} was about 80mg/100gram and 800mg/100gram respectively. Both substrates were simultaneously used to meet the energy requirements of the bacterial flora present.

As glucose and lactic acid were depleted (10^8 - 10^9 psychrotrophes/gram) the initial amount of total volatile N-compounds (23mg N/100gram) increased steadily and off-odours became perceptible.

The fresh LD samples with a high pH_{24} -value contained little or no glucose (4-20mg/100gram) and lactic acid (250-500mg/100gram). Consequently a depletion of these substrates occurred while bacterial counts were still low ($< 10^7$ psychrotrophes/gram).

After 13 days of cold storage the initial content of total volatile N-compounds (27mg N/100gram) amounted to 50mg N/100gram and spoilage odours were clearly detectable.

2:8

MICROBIAL DECONTAMINATION AND WEIGHT OF CARCASS BEEF AS AFFECTED BY AUTOMATED WASHING PRESSURE AND LENGTH OF TIME OF SPRAY

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Carcass beef traditionally have been washed by hand to remove foreign material such as hair, soil particles, and microbiological organisms that have contaminated the surfaces. These carcasses are inspected by the Food Safety and Quality Service to detect defects related to carcass cleanliness. Recent research and development of technology have emphasized automated machine washing. Under high pressures it is conceivable that water could penetrate tissue surfaces and be absorbed by the carcasses. Also, longer wash periods could possibly enhance water uptake by carcasses. The average shrinkage of carcass beef using good current practices was 1.3% at 20 h postmortem. The objectives of the research presently reported were to determine the effects of spray pressure and length of time washed on carcass weight and microbial flora.

Carcasses were obtained from 56 heifers that were fed a corn-corn silage diet to determine the effects of automated washing spray pressure (SP) of water of 2412 kilopascal (kPa) or 4134 kPa and chain speed (CS) of 3.9, 5.9 or 7.9 m/min on weight changes of carcass beef and microflora. Carcass beef sides were weighed before washing, 5 min after washing and 20 h after washing and storage at 0°C . Sides were sampled on the forequarter and hindquarter for microbial contamination before washing and 20 h after washing. Samples were cultured on violet red

bile agar with glucose (*Enterobacteriaceae*) and standard plate agar (aerobic) at 35°C for 24 or 48 h, respectively.

Interactions of SP and CS were not important sources of variation in side weight. Sides gained ($P < 0.01$) 0.83 kg (0.57%) weight during washing. However, the gain in side weight plus an additional ($P < 0.01$) 1.52 kg (1.04%) was lost during the 20 h storage period. Washing reduced ($P < 0.01$) *Enterobacteriaceae* counts by 1.52 logs (base 10) and aerobic counts by 0.87 logs. Reduction in *Enterobacteriaceae* counts was not affected by SP. However, a trend in greater ($P < 0.10$) reductions in aerobic counts was observed for the low SP compared to the high SP treatment. Bacterial counts were not affected by CS.

Research indicated that automated carcass washing was a useful procedure for reducing bacterial counts in carcass beef without affecting carcass weight.

2:9

ISOLATION OF YERSINIA ENTEROCOLITICA FROM PORK MEAT

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To evaluate the occurrence of *Yersinia enterocolitica* on pork meat, the Digastric muscle was selected as the sampling site. From 230 pork heads this muscle was removed. At the laboratory each muscle was homogenized and added to the enrichment broth Irgasan-Ticarcillin-Chlorate (ITC). After incubation plating was performed onto the selective agar plates Cefsulodin-Irgasan-Novobiocin Agar (CIN), Desoxycholate-Citrate-Mannitol Agar according to Saari and Jansen (YM) and Salmonella-Shigella-Desoxycholate Agar (SSD). Suspicious colonies were biochemically characterized.

Yersinia enterocolitica and related species were isolated from 89 (38.7%) of 230 pork heads. Positive samples contained following strains: biotype 1 (54 or 60.7%), biotype 2 (1 or 1.1%), biotype 4 (37 or 41.6%), *Yersinia frederiksenii* (5 or 5.6%) and *Yersinia intermedia* (1 or 1.1%). Nine samples contained 2 different types of *Yersinia*: beside the common biotype 4 in all these samples, 6 samples harboured also biotype 1, 2 samples *Yersinia frederiksenii* and 1 sample *Yersinia intermedia*. Pyrazinamidase activity was tested in the 292 strains isolated during the examination.

With the exception of all strains belonging to the biotype 2 and 4, pyrazinamidase test was positive.

The strains of biotype 2 and 4 were serotyped (by slide agglutination) as serotype O₉ and O₃ respectively.

Finally, an attempt was made to distinguish pathogenic from nonpathogenic strains by some recommended vitro tests such as calcium dependency at 37°C, autoagglutination at 37°C and latex agglutination.

2:10

PREVALENCE AND CONTROL OF *CAMPYLOBACTER JEJUNI* IN A TURKEY PROCESSING PLANT

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Poultry are by far the largest potential source of food-poisoning *Campylobacter jejuni*. In the present study equipment, turkeys and meat products were sampled during one month's production at a turkey processing plant. The purpose of this study was to determine the prevalence of *C. jejuni* and to identify the conditions that would control the spread of the organism during processing.

The types and numbers (in parentheses) of samples taken, were as follows: cloacal contents of birds (96); swabs from the defeathering machine (44); swabs from the internal cavity of carcasses after evisceration (96); breast meat (64), swabs from a 25 cm² area of skin (44) and swabs from the internal cavity of carcasses (44) after spray-cleaning; deboned meat (64) and swabs of the mechanical deboner (44); packaged, cooked products (201); packaged, sliced, cooked products (72). The selective medium used for the isolation of *C. jejuni* was Skirrow's agar, which when inoculated was incubated at 42°C for 48 h in a micro-aerobic atmosphere. The total viable count (TVC) of bacteria in packaged meat products was determined using plate count agar and saline as diluent. Plates were incubated at 30°C for 72 h.

C. jejuni was isolated from 67.7% of the turkey cloacal contents examined. The isolation rate from the defeathering machine was 4.5% and from the internal cavity of carcasses after evisceration, 32.3%. *C. jejuni* was not isolated from the remaining sites of

sampling. The TVCs in packaged meat products, both sliced and unsliced, were all less than 10⁴ organisms per gram.

It is concluded from these results that contamination of carcasses by *C. jejuni* is inactivated by spray-cleaning with water containing 40 ppm chlorine. The absence of *C. jejuni* from deboned meat and the TVC of packaged meat products suggest that hygienic handling procedures were in operation in the plant. Further, the cooking process (core temperature of 73°-75°C for 20 min) was adequate to inactivate *C. jejuni*. In conclusion, the findings of this survey show that good processing conditions, to yield a product with a TVC less than 10⁴ organisms per gram, can prevent the transmission of *C. jejuni* onto raw meat and cooked meat products.

2:11

BACTERIOLOGICAL QUALITY ASSURANCE OF PORCINE TONGUES.

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INTRODUCTION

Porcine tongues are mainly used for human consumption and generally incorporated in heat processed meat products. They are often visibly contaminated with blood, saliva, hair and dirt. Their keeping quality is limited. Bacteriological Quality Assurance (BQA) along the meat production-line has to rely on longitudinally intergrated Good Manufacturing Practices (GMP's). So far, BQA concerning porcine tongues has appeared to be insufficient. The purpose of the described investigation is (a) to obtain information on the bacteriological quality of porcine tongues in the different stages of the slaughtering process; (b) to elaborate GMP's for the production of porcine tongues.

MATERIALS AND METHODS

Line studies were carried out in sixteen slaughterhouses. Two investigators qualified the contamination grade of porcine tongues relying on fixed criteria, using a check list. At two plants a bacteriological linecontrol was carried out twice. At five stages of the slaughtering process i.e. after (a) bleeding (b) dehairing (c) evisceration (d) collection (e) centrifugation, samples of tongue mucosa were taken. The following colony counts per cm² were assessed: mesophilic aerobic colony count, *Enterobacteriaceae*, Gram negative bacteria and *Brochothrix thermosphacta*. Histobacterioscopic examination was carried out, in order to assess the numbers and location of microorganisms in tongues. Experiments on an advanced centrifuge process under standardised conditions were

involved in the investigation. The effects of time: (20s vs 40s), load: (10kg vs 15kg) and water expenditure: (20 l per min. vs 40 l per min.) on the bacteriological quality and cleaning efficiency were assessed. Aerobic colony counts and *Enterobacteriaceae* per cm² tongue mucosa were determined and histobacterioscopic examination of the mucosa was carried out.

RESULTS

After stunning and bleeding the mucosa of the tongues was contaminated with dirt, blood and mucus. Contamination of tongues with stomach contents and damage of tongues took place during dehairing and polishing. The grade of the visual perceptible contamination depends on: (a) filling grade of the stomach (b) type of machinery. Specific cleaning equipment in the slaughterline for tongues has hardly been developed. Cleanliness of the tongues was insufficient at 11 of the 16 plants. The highest bacteriological contamination of porcine tongues was found after bleeding. Mesophilic aerobic colony count, *Enterobacteriaceae*, Gram negative bacteria and *Brochothrix thermosphacta* were approx. 6.1, 2.9, 3.4 and 4.1 log₁₀ N per cm² respectively. Production stages reducing the contamination were scalding and the centrifuge process. The results of the bacteriological examinations were substantiated by the histobacterioscopic findings. By application of the advanced centrifuge process a 100 fold reduction of the transient flora viz. *Enterobacteriaceae* and in optimal cleanliness of the mucosa of porcine tongues can be attained. Considering economic as well as hygienic aspects a time, load, water expenditure ratio of 20 s/ 15 kg/ 20 l per min. is preferable. GMP concerning BQA of porcine tongues includes (a) slaughtering of pigs with an empty stomach only. (b) avoiding contamination during evisceration (c) application of a cleaning process, e.g. the centrifuge process.

2:12

THE BACTERIAL FLORA AND KEEPABILITY OF FINNISH FRANKFURTERS

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The effect of the following factors on keepability and bacterial flora of Finnish frankfurters was investigated: season (summer, winter), storing temperature (4°C, 7-8°C), storing time (1, 2, 3 and 4 weeks). The keepability was tested by evaluating organoleptically the flavour of frankfurters. The following microbiological determinations were made: Total count of bacteria, lactic acid bacteria, total count of anaerobes, staphylococci and micrococci, faecal streptococci, and coliforms. The number of thermotolerant bacteria of bacterial groups determined was examined by keeping plated dishes 2 hours at 60°C before incubation.

The season had no effect on the keepability of frankfurters. The flavour points of 4 week old sausages stored at 7-8°C in summer were significantly lower than the points of 4 week old sausages stored at 4°C in summer and at 4°C or 7-8°C in winter. The summer sausages stored for 4 weeks at 7-8°C were no longer suitable for sale. Season or storing temperature did not affect the bacterial counts determined.

Storing time caused more of an effect than the season or storing temperature on the keepability and on the counts of bacteria. The flavour points of 4 weeks old sausages stored at 7-8°C in summer were significantly lower than the flavour of respective 1 week old sausages. Usually the bacterial counts of 4 weeks old

sausages were significantly higher than the bacterial counts of one week old sausages.

The frankfurters contained about 1/10 thermotolerant bacteria in their total bacterial count.

It was shown that in particular special attention should be paid to the keeping temperature of frankfurters in summer.

2:13 CHOURIÇO SLAUGHTER

HEAT DESTRUCTION OF THE AFRICAN SWINE FEVER VIRUS IN THE PORTUGUESE "CHOURIÇO". TECHNOLOGICAL PARAMETERS.

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The portuguese "Chouriço" is a pork meat product whose source comes from the traditional country food. It is manufactured from fat and pork meat in which they are the main ingredients.

Traditionally it is a product that is smoked during a period from 15 days to one month, at low temperatures. The industrial processing ensures That temperatures utilized are above 60°C (quick process in a smoking house). The industrial processing consists on two steps: the first one, during 4-5 hours at temperatures above 60°C; the second one, during 12-18 hours, with temperatures between 40-50°C, in traditional smoking houses. This work shows that the thermal processing already mentioned and used by the portuguese industry can offer a lethal value which is good enough to ensure the sanity of this product concerning the virus of the african swine fever.

2:14

SURVIVAL OF SALMONELLA IN THE PROCESS OF LIVEX PRODUCTION AND ITS BEHAVIOUR ON THE LIVEX SURFACE

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During the production of a fresh fodder livex modified with whey, a reduction of Salmonella number takes place down to the level which ensures safe feeding of animals. When Salmonella gets onto the surface of a fresh brown livex basal and modified with whey, it starts to grow even at a temperature of 6°C, hence the livex has to be protected against the contamination with these bacteria, and stored at the temperature not higher than 4°C.

Heat penetration curves on the "critical point" have been determined concerning the thermal processing in several meat plant.

Five different technological experiments (chouriço) have been made in pilot plant with meat and fat from experimentally infected pigs. Different Thermal processing have been used. The lethal value has been calculated from the heat processing curves for an $F_{60^{\circ}\text{C}}^{4,5}$. Samples have been taken at different times and temperatures.

Afterwards, survival tests in vivo for the virus, have been carried on by intramuscular injection and oral intake.

It has been verified that the samples whose thermal processing reach "in the critical point" a lethal value above 14 ($F_{60^{\circ}\text{C}}^{4,5}$) did not produce infection in the test pigs. Below lethal values of 14 ($F_{60^{\circ}\text{C}}^{4,5}$) all test pigs died.