4. Microbiology and Hygiene

A Microbiological Method for Identifying Irradiated Frozen Chicken

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Investigations were carried out to identify irradiated frozen chicken using the synergistic killing effects of two types of combination treatments. In a model study, frozen chicken was contaminated with Lactobacillus plantarum, irradiated and heated in water bath. An irradiation dose of 3 kGy plus heating at 55°C for 15 minutes resulted in a 3 log reduction in the cfu/g in irradiated samples. Also, a dose of 4 kGy plus treatment with 8-8.5 % NaCl in aqueous solution synergistically reduced the Lactobacilli load by about 4 log cycle. Finally, the result of 8% NaCl plus 4 kGy on the natural Lactobacilli load in frozen chicken was significant to differentiate between irradiated and unirradiated frozen chicken. The NaCl treatment method could therefore be further developed to become a control method for identifying irradiated meat products.

Growth Profiles of *Vibrio* species Isolated from Danish Curing Brine

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Six different *Vibrio* species have been isolated from 4 commercial Danish curing brines used in the production of tank cured bacon sides. The six species were identified to be *V. proteolyticus, V. nereis, V. cambellii, V. logei, V. vulnificus* and *V. alginolyticus*, respectively, within *ca* 80 % homology. Addditional the growth of the different species were followed as a function of temperature (2, 5, 8, 12 °C), pH (6.0, 5.8, 5.5, 5.2, 4.9, 4,6) or NaCl-concentration (0.5, 4, 8, 12, 16, 20, 24 %) in a BHI-medium (Brain-Heart-Infusion) using a mirotiter-plate technique. These results showed in contrast to the normal nutritional characterization (criteria of *Bergey's Manual*) a further differentation of some of the isolated *Vibrio* species. The growth profiles as function of pH and to a minor degree the growth profiles as a function of salt concentration, respectively, indicate that at least some of the isolated species can be further differentiated into two separate groups. Especially *V. proteolyticus* and *V. nereis*, which were the dominating *Vibrio* species using the present isolation technique , were found fall into two seperate groups. One group, which can growth at pH > 5.8, and another group, which can growth in the somewhat unusual pH-area for *Vibrio* species, 4.9 < pH < 5.8. These observations are discussed in relation to these two species role during ripening and storage of tank cured bacon sides.

4

Prediction of Bacterial Penetration into Red Meat Carcass Tissues during Washing

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This study was conducted to determine the effect of line pressure on the penetration of bacteria into the tissue of red meat using Blue Lake, an insoluble dye, to simulate bacteria. The beef tissue surfaces studied were (1) exterior lean, (2) exterior fat, (3) interior body cavity and (4) cut tissue. Four strips (20 cm x 6 cm x 1.5 cm) of each of the tissues were placed on a polyethylene holding frame, moistened with distilled water and coated with Blue Lake. Next, the meat tissue was washed using the model Carcass Acquired Pathogen Elimination/Reduction (CAPER) system. The nozzle was a Spraying Systems Tee-Jet No. 5008, oscillated at 60 cycles per sec. Chain speed was 10 cm/sec. Volumes of 4.9, 8.3, 11.7 and 14.4 L/minute were sprayed at pressures of 690, 2070, 4140 and 6200 kPa, respectively. Depth of penetration of Blue Lake into control tissue was approximately 0.2 to 0.3 mm. Blue Lake penetrated control tissue significantly less than washed tissue. No differences were noted in effects of treatment between exterior lean or fat tissue with penetrati averaging about 1 mm at pressures of 690 and 2070 kPa. Interior surface tissue was more resistant to penetration than exterior tissue. Blue Lake penetrated cut tissue to greater depths than all other tissues. With the Blue Lake, weak tissue and breaks in the tissue were easily observed. Equations are presented describing penetration of the Blue Lake into the different types of tissue as a function of pressure.

Isolation of Campylobacter Species from Meat

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The occurrence of Campylobacter species in meats was investigated by using appropriate media for isolation and growth, and an incubation at 42°C in the presence of 10% CO_2.

In total 137 samples of meat and 61 samples of meat products were investigated, however, Campylobacter ssp. were not detected.

Pollution Control of Cooking Liquor from Abbatoir Offal and Meat Waste by Ultrafiltration: a Derived Source of Animal Protein and a Nutritional Broth for Microbial Culture Media

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The cooking broth of meat being a conspicous pollutant,and ultrafiltration an expensive operation,have justified an assay to upgrade the value of both the retentate and permeate.

Offal of slaughter operations were pressure cooked. The cold deffated liquor was filtered through a 10 000 Dalton polysulfone membrane. The liquor, derived permeate and retentate were analysed for DBO_5,solids in suspension, water content, ash, protein, fat and reducing sugars. The protein enriched retentate was incorporated into the cooked cake to formulate an intermediate moisture food (IMF) for dogs; the permeate was employed as a base nutrient broth for the following culture media: tryptone glucose extract plate count agar, azide dextrose broth (Difco Laboratories, USA), lactose broth and Baird Parker's tellurite glycine medium (Oxoid Ltd, UK). Tests were simultaneously conducted with the above mentioned commercial media on 20 bacteriological water and food analysis. Main results recorded were the reduction of DBO_5 and solids from average values of 900 mg O_2 and 4000 mg per liter, respectively, to 50 mg and traces per liter, respectively, in the permeate. The number and specificity of bacteria recovered was equivalent in the experimental and commercial media.

The pollution control of meat cooking waters can be achieved by ultrafiltration and the cost can be compensated by the utilization of the protein enriched retentate in IMF and of the soluble nitrogen rich permeate in microbiological culture media.

Effect of spices on the growth and acid production of Lactobacillus plantarum

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The aim of the present study was to investigate the effect of some natural spices and their extracts, which are usually applied in the manufacturing of bulgarian raw-dried meat produces, on the growth and acid production of the starter culture of Lactobacillus plantarum strain K_6.

The spices (cummin, savory, red pepper, black pepper, nutmeg, coriander) and their extracts were added in different concentrations in liquid medium. The medium used, with a pH of 6,0, composed meat extract, peptone, NaCl, glucose. The medium was inoculated with 24 hours old culture of L.plantarum strain K_6 with concentration $2{,}8.10^6$ cells/ml. The growth of L.plantarum after 24,48 and 96 hours incubation at 30 °C was estimated by counting on blood agar plates. Acid producing activity of strain K_6 in the liquid medium was determined by titration with 0,1 N NaOH. The amount of acid produced was calculated as µmol/ml lactic acid.

It was found that the natural spices cummin, savory, red pepper, coriander and mixtures contained cimmin+black pepper and savory+black pepper had a stimulatory effect whereas the black pepper and nutmeg had a small inhibitory effect on the growth of L.plantarum strain K_6. The spices extracts had the similar effect when used as 50 % concentration (v/v). The spices and their extracts (as 50 % concentration) used in this study had a stimulatory effect on the acid production of L.plantarum strain K_6. The stimulation of acid production by savory, cummin, red pepper and by the same spices extracts was the highest.

4

Microbiological Measurements of Hygiene in Danish Abattoirs

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The level of hygiene in three randomly chosen Danish pig abattoirs has been surveyed. The hygienic level w registered in different sites on the carcass and at different steps during the slaughtering process. At ea abattoir samples were taken at two separate occasions. The study includes a total of 175 carcasses.

Six sites on the carcass were studied: three sites on the rind surface and three sites on the meat surfac Sampling sites represented both high risk as well as low risk of contamination during slaughtering. The sa carcasses have been checked before evisceration, after final slaughtering, and after cooling. Samples have be taken using the double swab technique and analyzed for total plate count and Enterobacteriaceae.

On the rind surface, the highest total count was found on the inside of the hind leg. On the meat surface, t largest total count was found at the split sternum.

The carcasses are mainly contaminated in the scraping and polishing equipment. Between "before eviscerati and "after final slaughtering" there is a general decline in total count. The present study cannot totally accou for these findings. However, an increase in Enterobacteriaceae counts indicates that bacteria from the intestin tract, tools/equipment, or operators on the slaughterline contaminate the carcass.

In general, the Enterobacteriaceae level is found to be low. Enterobacteriaceae, as opposed to the total coun vary greatly from one abattoir to the next, both with respect to level and distribution on the carcass.

The Application of an Artificial Electric Field in Cold Storage of Pork and Beef Carcasses

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During slaughtering carcasses can be contaminated by direct contact with the skin or wi gastrointestinal content, by human or mechanical manipulation and by aerosols, carryi dustparticles and microorganisms. Since the keeping quality of meat is determined by t nature and the degree of initial contamination of the carcass surface, much attention should b paid to reduce sources of contamination.

Artificial electric fields have been described as beneficial for the indoor atmosphere especially in rooms where air contamination should be avoided.

The aim of this study was to evaluate the development of surface contamination of porc and be carcasses during refrigerated storage under the influence of an artificial electric field

This artificial electric field was obtained by the installation of an ECOSYSTEMS devic consisting in a vertical electrode-wall, covering one entire wall of the chilling roo Between this electrode-wall (positive electrode) and the rest of the chilling room (negati electrode) a potential difference was generated.

Under this atmosphere, 3 beef hind quarters and 3 pork hams were stored during 12 and 10 da respectively. Four times during storage, surface samples were taken to evaluate t development of aerobic psychrotrophes, Enterobacteriaceae, Pseudomonas spp., Yeasts and Mould The same experiment was performed in control circumstances.

The results indicate a very significant slower microbial development on carcasses under artificial electric field as compared with a control atmosphere. This effect was especiall obvious for aerobic psychrotrophes, Enterobacteriaceae, Yeasts and Moulds. The development Pseudomonas was retarded, but significance was lower, due to large numeric differences betwe carcasses.

These experiments gave evidence of the utility of artificial electric fields in fresh me processing, for controlling airborne contamination and its development on the meat surface.

Confocal Scanning Laser Microscopy of Contaminated Pork Muscle Surfaces

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The growth and survival of microorganisms on meat has been studied extensively, but little is known about the site of microbial activity on muscle. Microscopic observations using transmission (TEM) and scanning (SEM) electron microscopy, and to a limited extent light microscopy, have suggested that microbial contaminants are intimately associated with surfaces. Because the methods used to prepare samples for light or electron microscopy severely alter both the attachment substratum and microbial cell aggregates, interpretation of images can be difficult. Confocal scanning laser microscopy (CSLM) makes possible the observation of fully hydrated tissue samples that are only slightly altered during staining. We have used CSLM to study pork muscle surfaces contaminated naturally and inoculated with two common meat psychotrophs, *Pseudomonas fluorescens and P. fragi*. The samples were incubated at 4°C until cell densities reached 10^9 CFU/cm^2. A 3 minute stain in 0.1% acridine orange was sufficient for viewing of the muscle surface and associated bacterial cells. There appeared to be no preferred attachment or colonization sites, and microcolonies were observed both on the surface and between adjacent muscle myofibers. *P. fragi* microcolonies were large, raised and densely packed, whereas *P. fluorescens* spread over surfaces to yield smaller, flatter and less dense microcolonies. The space between cells in microcolonies is much greater in CSLM images than in SEM, This study confirms that surface colonization is an important parameter in the microbial ecology of spoilage bacteria on meat. In addition. other factors of importance in meat quality, such as fiber size and sarcomere lenght, can be readily observed and quantified using CSLM. The ability to observe the microstructure of meat in it's native state makes CSLM is a powerful tool for the study of meat quality.

Studies on Yeast Strains as Possible Starter Cultures for Meat Industry

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Yeast strains were investigated as possible starter cultures for use in the meat industry.

The cultivation conditions of the selected yeasts are examined in order to obtain higher biomass yield with food biological and technological properties.

Catalase, nitrate-reductase and peroxide-dismutase enzyme activities of the yeast strains were investigated to determine their specific effect on meat products during the process of ripening and how they influence the formation of stable colour, flavour and aroma.

The obtained yeast strains showed good technological properties and enzyme activity and can be recommended as starter cultures in meat industry.

4

The official bacteriological meat examination in Germany with special regard to clostridia - present status and tendencies

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Red meat of slaughtered animals is commonly contaminated with low numbers of clostridia (< 10/g), which probably dominate in the stage of vegetative growth. The official bacteriological meat examination in Germany has a long tradition of using liver broth to cultivate clostridia in samples taken from deep-lying muscle-tissues. This part of the cultivation procedure is not under discussion and has, until now, proved to be suitable.

However, does the inoculation temperature of the enrichment broth have to be "boiling hot"? The results of our pilot studies testing 6 strains of clostridia showed the best reisolation from liver broth tubes inoculated at only 20°C. There is no doubt that the method based on a 48-hour cultivation period must necessarily be a compromise. The official German cultivation technique for clostridia from red meat should be revised and new rapid methods should be established. Emphasis should be put on finding methods of detecting the fast growing species of clostridia. New and rapid cultivation media - for example the iron mild or the rapid - perfringens media - should be taken more into account.

Shelf Life Extension of Vacuum Packaged Vienna Sausages by In-package Pasteurization

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Spoilage of vacuum packaged vienna sausages by lactic acid bacteria results in considerable economic losses to local manufacturers. Consequently, the effects of in-package pasteurization and subsequent storage temperature variation on the microbiological shelf life and composition of predominant spoilage populations were examined.

In-package pasteurization of sausages in 80°C water for 20 minutes was followed by microbiological shelf life studies during storage at 7°C and 25°C (accelerated test). Time taken for total aerobic and lactic acid bacteria numbers to reach 5×10^6 g^{-1} was defined as microbiological shelf life. Bacterial populations predominating in all sample types were isolated and characterized.

Numbers of total aerobic and lactic acid bacteria were closely comparable and reached *ca.* 10^8 g^{-1} with increasing storage time for pasteurized and control samples at both storage temperatures. Pasteurization did, however, result in increased product shelf life. For pasteurized samples stored at 7°C, a *ca.* four-fold shelf life increase was recorded over untreated controls.

Lactic acid bacteria predominated on both total aerobic and lactic acid bacteria counts and leuconostocs and homofermentative lactobacilli collectively comprised the highest proportion of predominant spoilage populations in all sample types. While leuconostocs were recovered at lower frequency from samples stored at 25°C, the homofermentative lactobacilli were less affected by sample storage temperature. However, the predominance of the latter group decreased in pasteurized compared to control samples. Both pasteurization and 25°C storage of samples led to a diversification of lactic spoilage populations.

In control samples stored at 7°C, leuconostoc and homofermentative lactobacillus proportions reached maturity levels during the first 7 days of storage with minimal further changes up to 115 days' storage. For pasteurized samples stored at 7°C, proportions of leuconostocs increased and those of homofermentative lactobacilli decreased between 14 and 115 days' storage.

Effect of CO_2 packaging on the ropiness observed in meat products

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Effect of CO_2 packaging on the ropy slime production caused by lactobacilli strains was studied. Two ropy slime-producing strains A210 and C1 were used in the experiments. Forty cooked sausages were inoculated with the strain A210 and 58 sausages with the strain C1 and the sausages were packed separately in gas-impermeable bags of nylon polyethylene laminate. The bags were filled with CO_2. Sixteen and 24 vacuum packaged sausages inoculated with the same ropy slime-producing strains, respectively, served as controls. Two levels of inoculation (4,0 or 7.0×10^4 bacteria per sausage) were used for the both strains. After the 4 weeks' storage of the sausage packages at 8°C ropy slime production was evaluated and lactic acid bacteria population on MRS-S agar and pH were determined.

CO_2 packaging significantly reduced but did not totally prevent slime production. After small inoculation mean lactic acid bacteria populations were 6.20 and 6.94 in the CO_2 packages and 8.55 and 7.76 in vacuum packages when the strain A210 and C1 were used for inoculation, respectively. The difference was statistically significant in the case of the strain A210. The pH decrease was lower in CO_2 than in vacuum packages with both strains. When large inoculation was used statistically significant differences were observed neither in lactic acid bacteria count nor in pH decrease between CO_2 and vacuum packaging. Mean lactic acid bacteria counts were 8.73 and 8.14 in the CO_2 packages and 9.10 and 7.97 in vacuum packages after inoculation of the strains A210 and C1, respectively.

Heat and Cold Inactivation of Toxoplasma gondii in meat

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Tissue cysts of Toxoplasma gondii, which may be found in sheep, swine, goats or chickens, are inactivated by the proper exposure to heat or cold. Though the literature provides some temperatures for the destruction of the parasite, the industry and the consumer may wish to utilize less severe exposure temperatures for longer time periods. Pork from pigs infected with approximately 10,000 T. gondii oocysts of each of 6 to 8 strains, for a total of 60,000 to 80,000 per pig and spiked with infected rat brains, was subjected to a sequence of times and temperatures for the heat and cold inactivation of the parasite. A curve, Log T = 7.92 - 0.146t where T is the time of exposure and t is the temperature (C), characterized the heat inactivation of T. gondii ($r = 0.72$). A curve, Square Root (Hr) = 26.72 + 2.16 (C) characterized the cold inactivation of T. gondii ($r = 0.77$). These heat and cold treatments are less severe than those recommended for the inactivation of Trichinella spiralis in pork. The industry will be well served by additional research to develop a vaccine for cats, which are an intermediate host, and by additional research to define the effects of processing agents and procedures on the inactivation of T. gondii.

4

Direct Epifluorescent Filter Technique (DEFT) as a Rapid Method in Microbiological Quality Assurance of Meat and Meat Products - Experience in Practice

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DEFT has not yet been used in food microbiology in Czechoslovakia. The DEFT has been tested on samples of cured and raw ground meat, seasoned minced meat and some heat treated meat products (salami, liver paté salami).

The results obtained from the DEFT within one hour were satisfying. Good agreement between DEFT and SPC (standard plate count) was found for these products within the range of 10^4 to 10^8 g^{-1}. The correlation coefficients were 0.87 (raw ground meat), 0.82 (cured meat), 0.86 (minced meat), 0.81 (salami) and 0.78 (liver paté salami).

The conclusion was made that DEFT could be used as a screening method to ensure a short period for microbiological examination of raw meat and some meat products.

Hazard Analysis and Critical Control Point Programmes in Relation to Slaughter Hygiene

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Improved animal health and controls applied during all stages of animal production, transport, lairage, slaughter and distribution have in many countries eliminated some diseases once transmitted from animals to man. However, much human enteric disease is still associated with the consumption of food of animal origin, caused by organisms present in the gut flora of healthy animals and not detected by routine ante-mortem veterinary inspection. These organisms, initially present at low numbers, proliferate when food is incorrectly handled during processing, distribution or preparation. Traditional control by inspection to judge compliance with good practices has failed to improve hygiene at slaughter. Additional random microbiological testing is of limited value because of the problem of sampling sufficient units, and relatively slow and imprecise methods.

A more effective means of control is the hazard analysis critical control point (HACCP) system. Having identified hazards, their severity and risks of their occurrence, critical control points are identified and monitored. There is general agreement most of the microbiological hazards, and the critical control points in the slaughter of red meat animals have been identified. However, HACCP has scarcely been implemented in abattoirs. Why? There appear to be several reasons: 1. Widespread beliefs that there is no need to change the traditional approaches; 2. That HACCP has already been applied; 3. That hygiene can be left to "those who know best"; 4. A poor understanding of advances in microbiology and temperature monitoring; 5. That the consumer is no interested. These beliefs result from from misunderstanding of the principles of HACCP and a misplaced confidence in the efficacy of controlling hygiene by traditional inspectional procedures.

Even if the priciples of HACCP are understood, they may be difficult to apply in the abattoir e.g. slaughter operations may differ and objective monitoring of complex manual operations is difficult to achieve. The minimum requirements are that control measures are specified and monitored, and full records are kept.

Limited microbiological testing is a useful check that HACCP has been properly applied i.e. the identified CCPs have been controlled. Such verification tests may also reveal unexpected hazards or CCPs that were overlooked in the hazard analysis. There is need for rapid on-line tests, so that if contamination occurs, or exceeds specified levels, corrective action can be taken immediately rather than retrospectively.

Techniques that could have application include: 1. Measures of total micobial load: ATP, dye reduction, DEFT, flow cytometry - electical e.g. conductance/impedance; 2. Detecion of pathogens: immunology (ELISA), gene probes; 3. Monitors of careless slaughter e.g. spillage from the gut: automated VFA, thermography; 4. Monitors during chilling: temperature at the suface and in deep muscle, water activity at the carcass surface.

Influence of Aerobes on Volatiles Accumulating in Vacuum-packaged Beef

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Vacuum packaged (VP) beef stored at low temperatures shows a microflora dominated by lactic acid bacteria (LAB). If storage is prolonged souring will occur, spoiling the meat. However the production of the compounds causing souring occurs after numbers of LAB have reached their maximum. Hence simple counting of bacteria is of limited use in assessing the remaining storage life of VP beef. Using gas-liquid chromatography (GLC) the level of simple carbohydrate volatiles in the drip of VP beef was studied. However the expected patterns of accumulation were not seen. Compounds were seen to accumulate then disappear. To explain this observation microflora on the meat surface and the inner surface of the barrier bag were investigated; pseudomonads, total anaerobes, coliforms and yeasts were enumerated. Regression analysis between the populations on each surface showed significant relationships ($P<0.001$). Pseudomonads preferentially colonised the bag with log(tvc) on the meat being 0.6 times those on the bag. This population, given a low level of permeating oxygen, was thought to be responsible for the loss of volatiles by simply using them as a carbon source. Studies with pure cultures of LAB and pseudomonads investigated the accumulation of ethanol and lactic acid in VP model meat systems. It was seen that production by LAB was followed by consumption in the presence of pseudomonads, confirming the hypothesis that loss of the volatiles was due to aerobic metabolism.

Chemical and Microbiological Changes in British Fresh Sausage

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The shelf-life of fresh British-style sausages is determined by organoleptic changes produced by microbial metabolism. The SO_2 in sausages limits the types of organism that grow but there is substantial consumer pressure to remove SO_2 from products. If this is done, a different microbial population is found and spoilage occurs more rapidly. In order to control this growth, attempts have been made to influence the metabolism of the microbes using different carbon sources in the sausage formulation since it is known that different end-products with different flavour profiles are produced in culture media. At this stage of the study, some of the major volatiles contributing to the spoilage aroma of the sausage, previously detected in vacuum-packed beef and luncheon meat, have been determined by means of gas chromatography and mass spectrometry, and those contributing to the off-odour identified by GC-odourport analysis. Acetoin and isobutyric acid were found to play the most important role; 2-metyl butyric acid and isovaleric acid are of secondary importance, and diacetyl and acetic acid did not contribute greatly to the spoilage aromas. The extent of microbial growth registered in the sausages, namely Brochothrix thermosphacta and Pseudomonads are in accordance to previous studies on meat.

The utilisation of reducing sugars already present in a standard sausage formulation with and without SO_2 has been determined. The levels of reducing sugars are low (0.25% w/w) at the start but increase to about 0.4-0.5% after 3 days and then decline, the process being more rapid in the formulation without SO_2.

4

Botulinal Toxin Development in Beef Sausages Containing Decolorized Red Blood Cell Fractions

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Toxin production by *Clostridium botulinum* was studied in a model cured beef sausage containing intact and decolorized dried bovine b fractions. Fractions included: plasma, red blood cells (RBC), acetone treated RBC, enzyme treated RBC, and peroxide treated R Products were formulated with: beef chuck, 2.5% NaCl, 3% blood fractions, 156 μg/g $NaNO_2$, 550 μg/g sodium ascorbate, and 10% w Approximately 500 and 5000 spores/g of a mixture of 6 type A and B proteolytic strains of *Clostridium botulinum* were mixed in. Sam were vacuum packaged, heated to an internal temperature of 72° for 20 min, cooled, then stored at 28°. Samples were withdraw weekly intervals and analyzed for residual nitrite and neurotoxin. Iron levels (μg/g) for blood fractions were as follows: RBC (1 acetone/RBC (1496), peroxide/RBC (1406), enzyme/RBC (130), and plasma (63). Beef contained approximately 20 μg/g total Samples formulated with 3% dried blood fractions contained iron levels (in μg/g) of: RBC (94), acetone (71), peroxide (59), enzyme plasma (17), and controls containing no blood fraction (17). Residual nitrite levels were low throughout the entire trial period. cooking nitrite levels varied between 10-40 μg/g and fell below 10 μg/g in all samples by week 1. Time to toxin detection was in inversely related to inoculum size. Regardless of spore numbers toxin was detected earlier in samples containing higher levels of The order, from first to last, was: RBC, acetone, peroxide, enzyme, no blood (control), and plasma. Time to toxin is predictable by an exponential decay equation, derived in earlier research. Plasma appeared to offer some protection from botulinal toxin develop These results demonstrate that the method used to decolorize RBC fractions has a significant impact on the potential for growth toxigenesis by *Clostridium botulinum* when these ingredients are added to cured beef products.

Biochemical and genomic characteristics of *Micrococcaceae* from french dry sausages

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Fifty strains of *Micrococaceae* isolated from french sausages were characterized by using API Sta system, by determinating their resistance to antibiotics, by measuring some biochemical activities (production D and L lactate isomers, peptidases and lipases). These strains were susceptible to lysostaphine and so w classified in the genus *Staphylococcus*. On the basis of their biochemical characteristics most of them were easly identified to known species. To help their identification, DNA-DNA hybridizations were carried out. So strains do not share any homology with species frequently found in meat products :*Staphylococcus xylosus Staphylococcus carnosus* .

Effect of Chitosan-acetic acid Mixture on the Growth of bacteria on Non-vacuum Packaged Pork Stored at the Temperature of 10°C or 20°C

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Fresh pork and pork innoculated with Staphylococcus aureus, Salmonella typhimurium and Listeria monocytogenes were separately dipped for 10s in 1% chitosan-2% acetic acid mixture (CA mixture), 2% acetic acid solution (A solution) and sterile distilled water, and allowed to drip for 3-5 min before packing in polyethylene bag, then stored at 10°C or 20°C. Effect of CA mixture and A solution on the growth of various bacteria on fresh pork were determined, including examination of the total bacterial count, Pseudomonas, Enterobacteriaceae, Lactobacillus, Staphylococcus, Micrococcus, Enterococcus, S. aureus, S. typhimurium and L. monocytogenes and the antibacterial activity of CA mixture and A solution was compared. The results showed that during the entire experiment, CA mixture had relatively strong inhibiting effects on various spoilage and pathogenic bacteria, including lactobacillus. Inhibition of Enterobacteriaceae and Enterococcus was strogest by CA mixture. Although CA mixture was as effective in removing bacteria from the surface of fresh meat as A solution on 0 day, but effectiveness of CA mixture against bacteria was greater than by the A solution. The shelf life of the meat treated with CA mixture was correspondingly longer than of meat treated with A solution. It was found that CA mixture had much greater inhibition on bacteria at 10°C or 20°C.
Furthermore, the outgrowth of Clostridium in fresh meat was examined, the results indicated that CA mixturer as well as A solution had an inhibiting effect on these bacteria, however, with CA mixture the inhibition was stronger than with the A solution.

Meat products made of coarsely ground pork: Survival of lactic acid bacteria and pseudomonads after heat treatment

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The bacterial flora of the surface layer and the core of meat products made of coarsely ground pork was investigated at the moment of spoilage when stored at 7°C or 4°C. The dominating strains were isolated and their heat resistance was studied in APT-broth, on APT-agar and in coarsely ground, cured pork.
The main strains in the surface layer of the products were coccoid lactic acid bacteria the counts ranging from 3.5 to 7.8 log cfu (colony forming units)/g. The core of the products contained coccoid lactic acid bacteria, coccoid lactic acid bacteria and pseudomonads, or only pseudomonads as the main strains, the counts of ranging from 2.5 to 6.0 log cfu/g.
The strains isolated from the surface layer of the products survived only accidentally after heating for 15 min at 72°C in APT-broth. Most strains isolated from the core of the products survived after heating for 30 min at 72°C in APT-broth at least in three tests out of six. It must be pointed out that all pseudomonads isolated from the core survived after heating for 60 min at 72°C in APT-broth and often after heating for 15 min in coarsely ground, cured pork (core 72°C). The numbers of inoculated pseudomonads decreased when the experimental porks were kept at 4°C after the heating. This indicates that they probably do not constitute a serious spoilage factor in cooked meat products.

4

Botulinal Toxin Development in Beef Sausages Containing Decolorized Red Blood Cell Fractions

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Toxin production by *Clostridium botulinum* was studied in a model cured beef sausage containing intact and decolorized dried bovine bl fractions. Fractions included: plasma, red blood cells (RBC), acetone treated RBC, enzyme treated RBC, and peroxide treated R Products were formulated with: beef chuck, 2.5% NaCl, 3% blood fractions, 156 μg/g $NaNO_2$, 550 μg/g sodium ascorbate, and 10% wa Approximately 500 and 5000 spores/g of a mixture of 6 type A and B proteolytic strains of *Clostridium botulinum* were mixed in. Sam were vacuum packaged, heated to an internal temperature of 72° for 20 min, cooled, then stored at 28°. Samples were withdraw weekly intervals and analyzed for residual nitrite and neurotoxin. Iron levels (μg/g) for blood fractions were as follows: RBC (19 acetone/RBC (1496), peroxide/RBC (1406), enzyme/RBC (130), and plasma (63). Beef contained approximately 20 μg/g total i Samples formulated with 3% dried blood fractions contained iron levels (in μg/g) of: RBC (94), acetone (71), peroxide (59), enzyme (plasma (17), and controls containing no blood fraction (17). Residual nitrite levels were low throughout the entire trial period. cooking nitrite levels varied between 10-40 μg/g and fell below 10 μg/g in all samples by week 1. Time to toxin detection was in inversely related to inoculum size. Regardless of spore numbers toxin was detected earlier in samples containing higher levels of i The order, from first to last, was: RBC, acetone, peroxide, enzyme, no blood (control), and plasma. Time to toxin is predictable by u an exponential decay equation, derived in earlier research. Plasma appeared to offer some protection from botulinal toxin develop These results demonstrate that the method used to decolorize RBC fractions has a significant impact on the potential for growth toxigenesis by *Clostridium botulinum* when these ingredients are added to cured beef products.

Biochemical and genomic characteristics of *Micrococcaceae* from french dry sausages

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Fifty strains of *Micrococaceae* isolated from french sausages were characterized by using API St system, by determinating their resistance to antibiotics, by measuring some biochemical activities (production D and L lactate isomers, peptidases and lipases). These strains were susceptible to lysostaphine and so w classified in the genus *Staphylococcus*. On the basis of their biochemical characteristics most of them were easly identified to known species. To help their identification, DNA-DNA hybridizations were carried out. So strains do not share any homology with species frequently found in meat products :*Staphylococcus xylosus* *Staphylococcus carnosus* .

Effect of Chitosan-acetic acid Mixture on the Growth of bacteria on Non-vacuum Packaged Pork Stored at the Temperature of 10°C or 20°C

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Fresh pork and pork innoculated with Staphylococcus aureus, Salmonella typhimurium and Listeria monocytogenes were separately dipped for 10s in 1% chitosan-2% acetic acid mixture (CA mixture), 2% acetic acid solution (A solution) and sterile distilled water, and allowed to drip for 3-5 min before packing in polyethylene bag, then stored at 10°C or 20°C. Effect of CA mixture and A solution on the growth of various bacteria on fresh pork were determined, including examination of the total bacterial count, Pseudomonas, Enterobacteriaceae, Lactobacillus, Staphylococcus, Micrococcus, Enterococcus, S. aureus, S. typhimurium and L. monocytogenes and the antibacterial activity of CA mixture and A solution was compared. The results showed that during the entire experiment, CA mixture had relatively strong inhibiting effects on various spoilage and pathogenic bacteria, including lactobacillus. Inhibition of Enterobacteriaceae and Enterococcus was strogest by CA mixture. Although CA mixture was as effective in removing bacteria from the surface of fresh meat as A solution on 0 day, but effectiveness of CA mixture against bacteria was greater than by the A solution. The shelf life of the meat treated with CA mixture was correspondingly longer than of meat treated with A solution. It was found that CA mixture had much greater inhibition on bacteria at 10°C or 20°C.
Furthermore, the outgrowth of Clostridium in fresh meat was examined, the results indicated that CA mixturer as well as A solution had an inhibiting effect on these bacteria, however, with CA mixture the inhibition was stronger than with the A solution.

Meat products made of coarsely ground pork: Survival of lactic acid bacteria and pseudomonads after heat treatment

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The bacterial flora of the surface layer and the core of meat products made of coarsely ground pork was investigated at the moment of spoilage when stored at 7°C or 4°C. The dominating strains were isolated and their heat resistance was studied in APT-broth, on APT-agar and in coarsely ground, cured pork.
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Combined effect of packaging and lactic acid fermentation in increasing meat shelf-life.

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Lactic acid fermentation is a very successful method to decrease pathogens and spoilage microorganisms. The amount of lactic acid produced during a given fermentation, as well as the decrease in pH and competitiveness of inoculated microflora of homolactic bacteria depends on a large extent on carbon source, storage temperature, oxygen availability and animal species as substrate. The study was design to know the contribution of each factor to potential decontamination of beef and pork cuts, as well the depth at which lactic acid penetrates into the meat. Oxygen availability was modified by wrapping the meat samples in a semipermeable wrapping film or by vacuum packaging. Two inocula were applied to pork and beef samples and stored at 15 and 27°C during a total study time of 4 days. Results showed penetration of lactic acid up to 4 mm depth; pH decrease was more marked in pork samples. In both animal species, a considerable decrease of Pseudomonas and Brochotrix thermosphacta populations was observed. There was no significant differences between samples wrapped in the semipermeable film and vacuum packaged. It was assumed that, although homofermentative lactic acid bacteria proliferate in both types of packaging condition, heterofermentative bacteria population also increased, and these are the main responsible for spoilage in the studied conditions.

Fate of Listeria monocytogenes and Salmonella in Flaked Unwashed and Color-Modified Turkey

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The growth/survival of Listeria monocytogenes and Salmonella spp. in two thermally-processed turkey raw materials was investigated. Boneless, skinless thighs were processed with a Unicom 1000 Flaker to prepare conventional flaked unwashed turkey (FUT). A 4-Kg portion of FUT was washed successively with chilled (2°C) 0.03 M sodium phosphate buffers (pH 5.8, 7.4, and 8.0) to produce color modified turkey (CMT). Turkey raw materials were cooked in sterile 250-mL metal beakers to an internal temperature of 71.1°C in a 93.3°C water bath and cooled. Cooked tissues were inoculated with 3.0 log CFU/g of L. monocytogenes and 3.5 log CFU/g of Salmonella to simulate post-processing contamination conditions. Inoculated FUT and CMT samples were held at 4 and 20°C. Salmonella numbers fell slightly in cooked raw materials stored at 4°C for 21 days, but increased approximately 6 logs by 2 days in FUT and CMT held at 20°C. L. monocytogenes increased approximately 5 logs in samples held 14 days at 4°C. By 2 days at 20°C, L. monocytogenes increased more than 5 logs in both raw materials. Except for one sampling interval (day 7 tissues inoculated with L. monocytogenes), there was no difference (P>0.05) in microbial numbers between FUT and CMT. When comparing data of this study with those investigating the growth/survival of these L. monocytogenes and Salmonella in poultry products, it does not appear that FUT and CMT are unique in their ability to support the growth/survival of these pathogens.

Listeria Monocytogenes in Frankfurters and Ready-to-eat Sliced Meat Products

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The occurrence of Listeria monocytogenes (L.monocytogenes) in frankfurters and ready-to-eat sliced meat products was studied. Four categories of meat products were selected: a) cooked frankfurter sausages, b) raw, cured, smoked, sliced pork loin (a_w >0.95), c) cooked, cured, sliced, rolled sausages, and d) cooked, cured, sliced ham. A total of 304 samples were examined qualitatively as well as quantitatively shortly after packaging, and at the end of the declared storage life. At this point a test was made also for lactic acid bacteria.

Results: In category a) 4 samples (6%) were positive for L.monocytogenes at packaging date and 8 (13%) at expiry date; in category b) 18 samples (23%) were positive at packaging date and 17 samples (21%) at expiry date; in category c) and d) 8 samples (10%) were positive at production date and expiry date. With 3 exceptions, the quantitative tests were negative (less than 100 L.monocytogenes/g). The highest figure found was 4000 L.monocytogenes/g at expiry date in a frankfurter sausage.

Conclusion: Lack of growth of L.monocytogenes in the storage period can be explained by inhibitory effect of lactic acid bacteria. However, the finding of L.monocytogenes in cooked and ready-to-eat products is cause for concern since a naturally occurring inhibitory factor is not a reliable control parameter. This observation is supported by the fact that the lactic acid bacteria count was low in the 3 samples found positive by the quantitative test.

The combined effect of cooking and storing temperatures on the quantities of aerobic bacteria in cooked sausage

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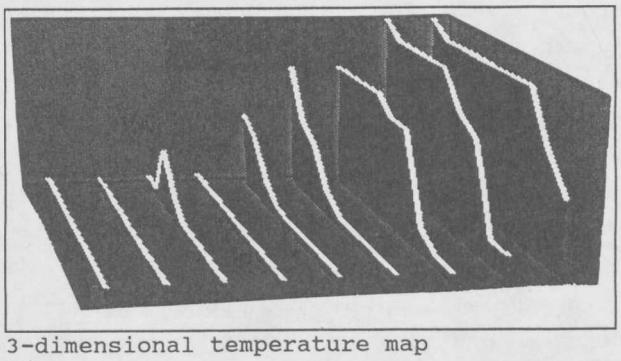
Using a plate type temperature gradient incubator (Gradiplate[R], Biodata Ltd., Helsinki) raw sausage material was exposed to 81 different combinations of cooking and cold storage temperatures according to a 9^2 factorial design. Cooking for 30 minutes in the range 54-69 °C was followed by 14 days cold storage between 1-11 °C under aerobic conditions. The numbers of naturally-present aerobic, coliform and lactic acid bacteria in the sausage material were determined by the agar dip slide method, and plotted in 3 dimensions against the corresponding cooking and cold storaging temperatures.



3-dimensional temperature map

In general low bacterial counts corresponded with high cooking temperatures and low storage temperatures, but the overall pattern of growth was not readily predictable. Therefore the 3-dimensional plots (maps) can be used as models to predict the probable numbers of bacteria of cooked and cold stored sausages. This temperature mapping study also confirms that avoidance of postcookery recontamination provides the method of choice for improving the microbiological quality of cooked sausages.

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The relative bacterial levels in different samplig sites of beef carcasses measured with the Ølgaard method

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The relative bacterial levels of beef carcasses in five sampling sites were monitored using the Ølgaard swab/agar plate method. The study was carried out during nine months (February - October 1990) in one finnish slaughterhouse. Five carcasses were sampled daily at the end of the slaughter line. The total number of samples was 3775 taken from 755 carcasses. The sampling sites were lateral round, flank groin, lateral forerib, shoulder and neck.

In the total material the most contaminated site was shoulder and the least contaminated was round. The means of the relative bacterial counts per sample of these sites were 89 and 5.5, respectively. In flank, forerib and neck the values were 54, 36 and 27, respectively. The monthly means of the relative bacterial counts in forerib and neck were between the values of shoulder and round during the whole sampling time. In flank, however, great variation of the monthly means of the relative bacterial numbers was observed during the last six months. The minimum was 18 in July and the maximum 450 in September. During that time the numbers exceeded twice the values of shoulder.

Selected characteristics of a bacteriocin from Lactobacillus sake 449

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The lactic acid bacteria have the potential to inhibit the growth of pathogenic and spoilage bacteria and the possibility exists of using them to improve the hygienic quality and to extend the shelf-life of different meat and meat products. Bacteriocins produced by lactic acid bacteria are also interesting to the meat industry for its possible uses as food preservatives, once they have been adequately characterized. We report in this communication some selected characteristics of a bacteriocin produced by L. sake 449, a lactic acid bacteri previously isolated from Spanish dry fermented sausages.

The antagonistic activity of L. sake 449, detected with L. fermentum CECT285 as the indicator microorganism, was evaluated in MRS and in a semisynthetic defined medium (SDM) with several supplements. The antagonistic activity was a growth-associated property, being detected and quantified when L. sake 449 was grown either at 8, 16, 25 and 32 ºC. The antagonistic effect of a concentrated culture supernatant of L. sake 449 was tested on various Gram-positive and Gram-negative bacteria. The supernatant was active against several lactobacilli, Staphylococcus and Listeriae, but none of the Gram-negative bacteria tested were inhibited, those included, among others, S. typhimurium and Y. enterocolitica. The antagonistic activity was bacteriostatic against the indicator microorganism. The activity was degraded completely by treatment with papain, protease II, protease XIV, pepsin and trypsin. However, the activity of the concentrated supernatants was resistant to heat, about 70% of the original activity remained after heating for 20 min at 100 ºC. When the concentrated supernatant was passed through a column of Sephadex G-150 we observed the formation of aggregates

Factors Affecting Growth & Survival of Salmonella in Dry Sausage made under Australian Conditions

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Fermented sausages receive no heat treatment and consequently must rely on other environmental conditions for their safety and shelf stability. In Australia, Salmonella has been the causative agent of food poisoning outbreaks associated with these products. Low pH and reduced water activity are the environmental factors which contribute significantly to the product's shelf life. Our investigations have found that both mechanism and rate of acidification are critical factors controlling the growth and survival of Salmonella. In Australia, it is not uncommon to manufacture salami using the approved food grade acidulant Glucono-delta Lactone (Gdl) as a means of rapid pH reduction. Alternatively starter cultures may be used as a means of reducing pH. The results of experiments designed to compare and contrast the effectiveness of starter cultures and Gdl have demonstrated that pH reduction brought about by lactic acid production by starter cultures is far more effective for control of Salmonella than the use of Gdl alone. Depending on the physiological state of contaminating Salmonella it is possible for growth to occur, when acidification is brought about by Gdl alone. In contrast pH reduction brought about by starter cultures results in no growth of Salmonella particularly when the pH is reduced to approximately 5.0 within the first twenty four hours. Additionally starter cultures proved more effective in the destruction of Salmonella. Certain types of starter cultures brought about a three log reduction of Salmonella numbers whereas Gdl alone effected only a one log reduction.

Effect of a Elastase from Alkalophilic Bacillus Strain on the Tenderization of Beef Meat

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Proteolytic enzymes such as papain, bromerain and ficin have been used as meat tenderizers. However, they often produce undesirable products, due to extensive degradation of the myofibrillar proteins. In this study, we evaluated the ability of a new elastase produced by alkalophilic Bacillus sp. strain Ya-B to tenderize tough cuts of meat.

The elastase, a new type of alkaline serine protease, was purified easily from the culture broth. First of all, we investigated its elastolytic activity using elastin-orcein as substrate. The specific activity of the enzyme was 60-200 times higher than other proteases.

Secondly, the mode of myofibril degradation was assessed by using SDS-polyacrylamide gel electrophoresis after incubation of intact samples with enzymes or without enzyme. Elastase treatment resulted n little proteolysis against isolated myofibrillar proteins, in contrast to papain which degraded most of proteins, especially myosin heavy chain and actin.

Furthermore, in order to examine the effect of enzymes on tenderness, mechanical hardness was measured by a rheometer. When each enzyme solution was injected to the beef thigh muscle, the force required to cut the muscle fiber was reduced in enzyme-injected samples.

These data indicated that the elastase are promising as a ideal meat tenderizer.

4

Meat Inspection and Microbiological Diagnosis on Local Anthrax of Pigs

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A quick and accurate procedure for pig anthrax diagnosis useful for the meat processing industry was developed during over thirty years of practical experience and lecture of th auther,and was applied to about one hundred anthrax cases.It was also based on the knowled of the epidemiological behavior of local anthrax seen during slaughter,and the spectific property of pathological changes,as well as ante mortem laboratory diagnosis.Using this pr cedure the accuracy of meat inspection and therefore the health quality of the meat can increased.

The paper is divided into three parts:1.The epidemiological characteristics of anthrax. 2.T kinds of pathological feature of local anthrax and the principle symptoms seen during me inspection.3. The laboratory diagnosis.For the latter three topics are discussed:a)The for of local anthrax and it's culture features.b)The quick diagnosis at post morten inspectio (pre-diagnosin).c)The laboratory diagnosis(i.e.the definite diagnosis).

In this paper,the authers put forward some new practical and theoretical views on local a thrax,which can prove useful as reference and in discussions in meat production and teachi

Salmonellae among animals slaughtered in Assiut, Upper Egypt.

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This study was planned to determine the incidence and serotyping of Salmonellae in animals slaughtered Assiut abattoir. 595 specimens were collected under sterile conditions from apparently healthy animals (5 buffaloes & 35 cattle) subjected to slaughter. The prepared sample were transferred to enrichment broth (Selenite F broth & Tetrathionate broth) and incubated at 43°C for 48 hours. Loopfulls from each sample were streaked on Salmonella shigella agar "SS agar" and Xylose lysine desoxychoate agar "XLD agar" and incubated at 37°C for 24 hours. Suspected colonies (non-lactose fermenter) were subjected to biochemical and serological identification.

Salmonellae were frequently isolated from carcasses of buffaloes & cattle with percentages 12 & 5.7 respectively. Two serotypes, Salmonella tshiongwe and Salmonella rissen could be identified. The obtained results indicated that XLD agar gave better results for isolation of Salmonellae.

Proper hygienic measures were recommended for reduction of Salmonella in food of animal origin.