The microbiology of vacuum-packaged pork.

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In studies of the storage characteristics of vacuum-packaged pork, it was observed that, provided the pH of the lean was less than 6.0 and the oxygen permeability of the packaging film was low, the bacterial flora which developed on the lean surface during storage at 0°C was composed almost entirely of psychrotrophic lactic acid bacteria (typical population 5 x $10^7/cm^2$). However, the relative proportions of the various components of the microbial flora varied not only with the packaging film permeability but also with meat pH and the storage temperature. At 5°C the Gram-negative bacteria were a considerably larger proportion of the total flora than was the case when vacuum-packed pork was stored at 0°C. On meat of high pH (>6.0) increased growth of both Gram-negatives & Brochothrix thermosphacta occurred, and these organisms reached populations in excess of $10^6/cm^2$ and became significant in spoilage. Under any of the conditions studied, the composition of the flora present on fat and skin surfaces was qualitatively similar to that found on the lean.

The shelf-life of this product has been estimated using an analytical trained taste panel. When pork of pH 5.6-5.8 was stored at 0°C in film with an oxygen permeability of ca. 25 ml/m^2/24h/atm (measured at 25°C and 98% r.h.) spoilage was caused by the development of an "off" flavour which became significant after ca. 6 weeks storage. Meat of high pH spoiled more rapidly. At 5°C, the shelf-life of high pH pork was usually terminated by the appearance of visual spoilage (greening) which developed after 2-3 weeks storage. The high incidence of meat of pH greater than 6.0 presents a major problem in obtaining a satisfactory shelf-life for vacuum-packaged pork. Its' shelf life will be discussed in relation to that of beef stored under similar conditions.

5:2

The safety of Basturma, an Armenian type of dry beef, with respect to Salmonella

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In 1982 an outbreak of human salmonellosis occurred in California as a result of consumption of Basturma, an Armenian-type dried beef product. This immediately raised the question about the safety of this and other ethnic speciality meat products produced by small plants and distributed frequently to many parts of the country. This particular product, very popular in Eastern Mediterranean countries is made in the USA by curing the whole Semitendinosus muscle with injected brine (NaCl and $NaNO_2$), and dry salted at room temperature for 3 - 5 days. The product is then washed and dried for 3 - 4 days, next pasted with spices, dried for a further 3 - 5 days and then stored in the refrigerator until used.

In this study we first determined the survival of three Salmonella serotypes in the meat brines and the product made under traditional methods, and then developed thermal processing conditions which assured both Salmonella destruction and preservation of product characteristics. The experimental findings demonstrated the following:

1. Salmonella levels of 40-270 cells/ml meat brine (25% salt) decreased only slightly after 8 days at 4°C. The pathogens were not detected in the brines after 27 days at 4°C.
2. Curing the meat for 6 days at 4°C (final brine level: centre 7-9%, surface >15%, pH 5.4) gave a 10-fold reduction in the numbers of salmonellae. Dehydration (brine in centre >10%, surface >18%) caused another 0.68 - 0.82 decimal reduction (DR) and drying after pasting 0.04 - 0.18 DR. The total process caused 1.68 - 2.10 DR demonstrating the potential Salmonella survival during commercial processing without heating. Injection of 1% glucose into the meat did not alter the results significantly.
3. Heating the cured meats to an internal temperature of 51.8°C within 6 hours and dehydrating for 3 days caused >4.45 DR to the inoculated salmonellae. In studies on the effect of the individual processing steps we found that curing caused 0.96 - 1.10 DR, heating to 53°C 2.93 - 3.20 DR, dehydration for 4 - 6 days at room temperature 0.98 - 1.48 DR, and dehydration after pasting with spices 0.43 - 0.57 DR.
4. Incorporation of 0.8% acetic acid to the paste (pH 3.4) reduced cured meat surface contaminating Salmonella by a rate of 0.8 DR/day of drying.
5. Developed technology has minimized sufficiently the Salmonella risk without altering product acceptability.

5:3

Effect of vacuum aging and display on lipid oxidation of subcutaneous fat and three layers of beef longissimus

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M. longissimus steaks from 32 steers finished on four nutritional regimens (grass, short, long and forage fed) were used to study the effect of vacuum aging and display (5 days continuous under 1076 lux Deluxe Warm White light at 3° C) on muscle lipid and subcutaneous lipid TBA value determined by a fluorometric method. All steers were initially on brome and bluestem grass pasture supplemented in winter with alfalfa and protein. Grass fed steers were slaughtered directly off of pasture in September. Short fed steers were fed 80% concentrate (cracked corn, alfalfa haylage and supplement) and 20% corn silage an added 49 days and long fed an added 98 days. Silage fed steers received 60% corn silage and 40% concentrate for 98 days.M. longissimus (1st to last lumbar vertebra location) was removed from chilled carcasses, equally divided into 2 halves and the posterior half was vacuum aged at 1°C for 21 days. One steak from the anterior half was immediately sampled (fresh pre display) and the other was displayed for 5 days continuous under 1076 lux Deluxe Warm White lighting at 3°C prior to sampling (fresh post display). The vacuum aged half was treated as above (vacuum pre display and vacuum post display). Post display steaks were separated into 3 layers of equal 8.5 mm thickness (top, middle and bottom). Subcutaneous fat was separated into inner and out layers. Data were analyzed by analysis of variance with mean separation determined by the least significant difference procedure. Display increased M. longissimus and subcutaneous fat sample TBA values by 2 to 5 times their pre display values. No difference ($P>.05$) was detected at pre display between fresh (TBA value = 0.078) and vacuum aged M. Longissimus (TBA value = 0.100) except in the short fed group. At post display, vacuum sample TBA value was higher ($P<.05$) than fresh in every nutritional regimen and for the averages (vacuum 0.435 and fresh 0.231). Post display values showed bottom layer TBA value to be highest ($P<.05$) followed by top and middle in the short and long fed groups. This result indicated a greater combined effect of light reflected from the bottom of the display case and of catalysis by heme pigments and exudate in bottom layer as compared to the effect of direct light alone on the top layer. For vacuum samples the top layer TBA value (average 0.552) was highest ($P<.05$) followed by bottom (average 0.486, $P<.05$) and middle layer (average 0.265, $P<.05$), respectively. The top layer, directly exposed to light, exhibited greatest oxidation while the indirectly exposed bottom layer was less oxidized. Layer effect likely would be more pronounced if very thin surface layers could have been sampled. Our 8.5 mm thick layers likely contain varying stages of autoxidation. Subcutaneous fat samples showed higher ($P<.05$) TBA values for vacuum than for fresh samples. The fat sample TBA values were close to or higher than reported threshold TBA values of 1.0 and therefore may present flavor problems depending on proportion of fat consumed from a steak. No difference ($P>.05$) was found between the two layers of subcutaneous fat.

5:4

Hot-boning of pork - A microbiological evaluation of different packaging principles

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Hot boning is a new technique which has many potential advantages including the reduction of cooling space and refrigeration energy, increasing cut yield and facilitating centralized processing. However, the microbiological advantages (shelf-life) and disadvantages (hygiene), particularly in connection with packaging, are less well-known.

The aim of the present investigation has been to find out whether hot-boning of pork involves increased hygiene risks and to examine the effects of different gas packaging systems on the microbiological shelf-life of packaged hot-boned pork.

Top loin roasts of pork, hot-boned and cold-boned, were packaged under vacuum, both in a mixture of 90% CO_2 + 10% N_2 and in 100% CO_2. The packaged pork was stored at 4°C for 20 days, after which time the packages were opened and the pork was subsequently stored in air for 3 days at 4°C. Gas analysis of the packages and microbiological analysis of the pork were carried out.

Except for the total load of organisms, no general differences in the composition of the microflora, the generation of CO_2 from the meat, the drip in the packages and the meat colour could be observed between hot-boned and cold-boned pork. The initial bacterial load differed by about 2 log units between pork samples taken from the two boning procedures. This difference was maintained throughout the storage period. However, the packaging principles strongly affected the shelf-life of the meat. The quality promoting effect, in terms of total count and composition of the microflora of the different packaging systems, was increased in the order of vacuum $<$90% CO_2 + 10% N_2 $<$100% CO_2.

Thus the total count after 20 days of storage was 2-3 log units lower in 100% CO_2 than in vacuum. Furthermore, the microflora in 100% CO_2 was completely dominated by Lactobacillus spp, while Lactobacillus spp only comprised 50-60% of the microflora on vacuum-packaged pork (30%-50% Enterobacteriaceae).

All samples of hot-boned pork had a fairly reasonable bacterial status after being subsequently stored for 3 days in air (total count $\leq$7.0 log units/cm^2). Of the cold-boned samples only those previously stored in 100% CO_2 were acceptable (6.5 log units/cm^2). After air storage, only pork stored in 100% CO_2 still showed complete domination of lactic acid bacteria, irrespective of the boning method. - No pathogenic bacteria were found in any of the pork samples.

5:5

The validity of the TTT-concept on the shelf lives of chilled, cured meat products

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Various types of cured meat products, a luncheon meat product (A), smoked and cooked pork loin (B) and unsmoked pork fillet (C) were sliced and vacuum packed. All types were divided into 5 groups. Three of the groups were stored at constant temperatures, -3.5°C, +2°C or + 4°C. The last two groups were stored at temperatures of either -3.5°C or +4°C but were transferred from the one temperature to the other every week. One group was stored at the low temperature in odd weeks, the other in even weeks. Shelf lives were assessed by sensory evaluations, supplemented with bacteriological analyses.

The fat content in products A, B and C was about 3%, 25% and 4%, respectively, and there was 4.4, 5.1 and 4.8 g salt per 100 g water, respectively. These products are labelled with a "best before" date of 4 weeks at a max. storage temperature of 5°C.

For the uncooked product (C), the initial count was 400,000 per g, rising to 10 millions in 1 week and to 100 millions in 2 weeks at +4°C. Taste and smell were judged unacceptable after 4-5 weeks, again proving the bad correlation between organoleptic quality and the total number of bacteria in vacuum packed meat products. Products A and B were cooked; an initial count of about 8000 was found. After 4 and 3 weeks at 4°C, respectively, the total count was about 1 million; the organoloptic quality was judged unacceptable after 6-7 weeks. The bacterial flora in most samples consisted mainly of lactobacilli; Brochothrix thermosphacta was found occasionally. At -3.5°C, the microbial growth and the decrease in organoleptic quality was slower than at +2°C or +4°C, but in some samples at -3.5°C the surface of the sliced meat was moist, presumably a result of a partial freezing.

No differences were found between the two groups which had been stored at alternating -3.5°C and +4°C, neither in bacterial numbers, nor in organoleptic quality. This indicates that the TTT-theory (time, temperature, tolerance), used in shelf life calculations for frozen foods, might be valid for some chilled foods, meaning that the effect of different time-temperature episodes on remaining shelf life is cumulative, regardless of the sequence of the time-temperature episodes. The use of -3.5°C or even colder in production and wholesale storage rooms and during transport might make it possible to improve shelf life and the quality of these products. Further studies on the commercial use of deep-chilling (super-chilling) have been initiated.

5:6

Effects of packaging, processing and formulation on spoilage of pasteurized meat products

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Chub packed luncheon meat (i.e. emulsion cooked in a plastic casing) was used as a model system to identify the effects of product and processing variables on the microbiological stability of pasteurized meat products protected from post-cooking contamination. When commercially prepared chubs were cooked in a water bath, with minimal cooking (pasteurization value for reference temperature 70°C, P_{70}=40) the surviving microflora (10^3/g) was dominated by species of Lactobacillus, Brochothrix and Micrococcus but with more intensive cooking (P_{70}=105) the flora (10^2/g) was composed of Bacillus and Micrococcus species. On storage at an abusive temperature (25°C), chubs with P_{70} values between 40 and 90 developed a Streptococcus spoilage flora; those with P_{70} values between 105 and 120 developed an initial Bacillus flora which was ultimately displaced by Streptococcus; while those with P_{70} values of 135 or above developed a persistant Bacillus flora.

The Bacillus component of the spoilage floras, B. licheniformis, grew only at the surface of commercial chubs, growth developing most rapidly under casing seams and end seals. Packaging in casings of high oxygen permeability accelerated growth; storage under hydrogen suppressed it. Experimentation with B. licheniformis cultures in liquid medium (TYG broth) showed that spore germination, monitored by loss of heat resistance, did not require oxygen but that trace amounts of oxygen were required for outgrowth. Nitrite did not enhance spore destruction or inhibit aerobic growth but did inhibit anaerobic growth. NaCl reduced heat damage and enhanced spore germination. Under anaerobic conditions germinated spores became moribund. Although nitrite and salt enhance microbiological stability under anaerobic conditions, their effects are only transitory during prolonged storage of chubs at abusive temperatures because of oxygen permeation through the plastic casing.

The Streptococcus component, S. faecium, grew throughout minimally cooked chubs but only at surfaces of those subjected to more intensive cooking. In liquid medium, NaCl protected against heat damage but retarded recovery of heat-damated cells. After recovery, growth rates were unaffected by NaCl at concentrations present in chubs. Nitrite had no effect on the cells' survival of heat treatment but reduced the NaCl-mediated inhibition of recovery. The resistance of temperature-abused luncheon meat to streptococcal spoilage is therefore due to a prolonged heat-induced, salt-maintained pregrowth recovery phase rather than inhibition of growth of undamaged cells.

5:7

Effects of storage in vacuum packages, with CO_2, on the shelf life of beef mince - microbiological observations

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The suitability of vacuum packaging for the storage of beef mince over a period of several weeks was investigated. Forequarter meat was reduced to pieces the size of trimmings and vacuum packed in the presence and absence of solid CO_2 (2g/kg meat). Some meat was minced prior to packing and again solid CO_2 was added to half the packages. Shelf-life was investigated by unpacking the meat, mincing if required, and placing aliquots in standard overwrap packs covered with a high-transmission film. Storage and display were carried out at 1-2°C to allow clear resolution of microbial trends. Storage was for 0, 2, 4 and 6 weeks and display samples were analysed after 0, 3, 5 and 7 days for total aerobic (TVC) and anaerobic (TAC) bacterial counts.

Colour was studied using a recording reflectance spectrophotometer. The pH and extract release volume (ERV) of the meat was also studied. The experiment was carried out twice and the results subjected to analysis of variance to determine significant interactions. It was seen that the TVC was affected by storage, display and CO_2 addition and that significant interactions ($P<0.001$) occurred between these parameters. The TAC was affected similarly to the TAC but was also affected by whether the meat was packaged as mince or trim ($P<0.001$). Meat packed as trim has a lower TAC than the packaged mince.

The CO_2 addition reduced microbial growth in the vacuum packages but after 4 weeks of storage this difference was much reduced as the TAC had by then reached maximum numbers, 10^6 - 10^7/gram, and almost no subsequent growth was seen. However packs with CO_2 showed consistently lower TAC than ordinary packs.

The CO_2 also affected the TVC and this effect was most marked after 2 weeks storage. Packs containing CO_2 showed almost identical TVC to the control display packs which had not been stored. Thus 2 weeks of storage had been obtained with no adverse effects, due to the aerobic spoilage organisms, on the shelf life. In packs stored for 2 weeks in the absence of CO_2 the TVC rose rapidly on display, reaching 10^6/gram after 3 days, whilst packages containing CO_2 took 7 days to reach this figure.

In samples stored for 4 and 6 weeks the TVC remained quite stable at about 10^6/gram possibly due to inhibition by the anaerobes. The colour analyses showed beneficial effects due to CO_2 addition and indicated that the meat was best minced after storage.

Overall vacuum packaging was seen to be a suitable method for storing beef intended for the production of mince and that the meat was best packed in as large pieces as possible and with the addition of solid CO_2.

5:8

Scientific basis for selecting storage conditions for encased meat products

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The scientific basis of the theory of meat storage is presented. In meat storage there is a trend towards the equilibrium state with the environment. Thus the chemical potentials and the specific mass capacity of meat products, packages and the environment must be equilibrated. It has been shown that the hygroscopic properties of meat products, packages and sausage casings depend upon their porosity. The latter has been determined with a vacuum capillarimeter. In case of natural casings, pore radii are important in the range of 4 to 100 mc.

Knowing pore distribution as related to the radius, it is possible to model casings and packaging materials having desired hygroscopic properties.

5:9

Inhibition by a lactobacillus of the growth of Brochothrix thermosphacta in mixed culture.

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Brochothrix thermosphacta and Lactobacillus L13 were grown in pure and mixed culture, both on meat and in glucose-limited continuous culture in a chemostat. Both organisms grew well on beef of high pH (6.5-6.7) stored at 5°C. Under aerobic conditions, the maximum population of each organism exceeded 10^8/g in all cases, both in pure and mixed culture. Under anaerobic conditions the population of the lactobacillus reached 10^9/g in both pure and mixed culture. In contrast, whilst the population of B.thermosphacta exceeded 10^8/g in pure culture, its growth in mixed culture was inhibited. Starting at an initial population of ca. 10^4/g, it reached a maximum of ca. 6 x 10^6/g and then declined on further storage.

When grown in continuous culture at 25°C, Lactobacillus L13 reached populations of 10^8-10^9/ml under both aerobic and anaerobic conditions. This occurred not only in pure culture but also in mixed culture with B.thermosphacta. In pure culture, B.thermosphacta achieved steady-state population densities in excess of 10^8/ml under both aerobic and anaerobic conditions of growth. However in mixed culture with the lactobacillus its population was dependent upon oxygen availability. Under aerobic conditions, steady-state populations in excess of 10^8/ml co-existed with similar populations of the lactobacillus, but when conditions were made anaerobic its numbers dropped to about 10^6/ml and then stabilized. However, B.thermosphacta was never eliminated from the culture and it continued to co-exist with the lactobacillus even though this latter organism was always dominant under anaerobic conditions of growth. The affinity of each organism for the substrate glucose (K_s glucose) was determined. For B.thermosphacta the K_s values were ca. 0.1 mM and 0.6 mM under aerobic and anaerobic conditions respectively. For the lactobacillus K_s was ca. 0.1 mM under all conditions.

The results of the chemostat experiments in this study are consistent with population control by competition for substrate (glucose). Since similar growth patterns were seen on meat, competition for substrates may also be a controlling factor in that situation. Reports in the literature suggest that lactobacilli inhibit the growth of B.thermosphacta on packaged meats due to the production of antimicrobial agents. Further, environmental factors such as muscle pH and packaging film permeability have been shown to control the population levels attained by B.thermosphacta on vacuum-packaged beef. Thus, when the growth of B.thermosphacta is inhibited in the presence of lactobacilli, a number of mechanisms may be operating.

5:10

Conditions for the use of lactic acid as a decontaminant in the meat industry

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An attempt was made to interrelate the data obtained in experiments, conducted by our Department, along beef, veal and pig slaughter lines, using lactic acid (LA) for the decontamination of carcasses, cold and hot boned primal cuts, slaughter byproducts and butcher's knives.

First and foremost it was observed that provided Good Manufacturing Practices are strictly followed the microbial load of carcass surfaces will be substantially reduced. LA-decontamination may effect an additional reduction. Since in the early post mortem period bacteria are not yet attached to the meat surface LA-decontamination should preferably be applied to the hot carcass. It was demonstrated that, dependent on mode and duration of application, LA sprays, not exceeding 1% v/v (beef), 1.25% v/v (veal) and 1.5% v/v (pork) resulted in acceptable carcass colour scores. Blood spots, which are particularly prone to discoloration by lactic acid application should be removed at an early post mortem stage e.g. by strong showering. The difference in surface pH between LA-treated and control carcasses disappeared within 72 hours post mortem. Veal longissimus chops treated with LA solutions up to 2% v/v were not identified by a consumer taste panel as significantly different from controls.

The "immediate" bactericidal effect of LA-decontamination for beef, veal and pig carcasses as well as for pigs liver and veal brain, amounted to approx. 1.5 log cycles for the mesophilic colony counts, strongly dependent on substrate and conditions of decontamination.
In addition a "delayed" bacteriostatic effect was observed during storage which is probably the result of a prolonged lag phase of acid-injured micro-organisms surviving lactic acid decontamination.
Ecological surveys revealed that LA resulted in a shift towards a Gram positive bacterial association acting as an antagonist of enteropathogenic Gram negative bacteria.
Electrostatic application of LA solutions may contribute to limiting the amount of LA needed for effective decontamination.

By adding 2.7% v/v LA to the spray water of a specially designed disinfection unit for butchers knives a reduction in mesophilic colony counts was effected at 45° C which exceeded that achieved by conventional sanitizers at 82° C.

5:11

The effects of lactic acid decontamination and frozen storage on the keeping qualities of calf brain

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In two experiments the effects of lactic acid decontamination (LAD) and frozen storage on the bacteriological condition of calf brain were investigated.

The first experiment included 80 calves, whose brains were extirpated manually after splitting the occipital bone with an axe. Upon removal, 40 brains were sprayed with 1.25% v/v L-lactic acid, while forty brains remained untreated. At day 1, from 20 brains of each group, cone shaped samples of 10 g were excised at undamaged sites of the hemispheres and at the site of impact of the captive bolt. After 8 days of storage at 3 ± 1° C in polystyrene trays the 20 remaining brains were sampled. Bacteriological examination included Aerobic Colony Count at 30° C (ACC-30) and 4° C (ACC-4), mesophilic Enterobacteriaceae Colony Count (EC), Lactobacillaceae Colony Count (LC) and Lancefield-D-streptococci Colony Count.

For both locations and with regard to all parameters examined LAD resulted in significantly lower bacterial counts at day 1 as compared with controls ($p < ,025$). However, differences were slight particularly at the damaged locations where a reduction in ACC-30 and ACC-4 of only 0.3 log units was effected. With the exception of ACC-4 at the site of impact of the captive bolt which was 7.0 log and 7.5 log for treated and control brain respectively, bacterial counts were no longer significantly different at day 8. Moreover LA treated brain exhibited an unacceptable discolouration. From these findings it was concluded that LAD is unsuitable for a sufficient extension of the storage life of calf brain.

A second experiment involved the aseptically removed brains of 20 mechanically stunned claves. Ten brains were sampled at day 1 whereas brains of 10 other calves were stored at -40° C for 7 days, whereupon they were allowed to thaw for 1 day at 3 ± 1° C. At day 9 these brains had equally low bacterial counts as at day 1 although thawing loss was somewhat higher (5.1%) than the weight loss of cooler-stored controls (1.2%).

It is concluded that in view of the susceptibility of calf brain to bacterial spoilage, freezing should be taken into consideration as an effective means to prevent growth of bacteria that will lead to deterioration.

5:12

Bacteriological control of baby food poultry products

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Bacteriological control during the hygienic processing of raw chicken meat and in the production of poultry-based baby foods (in either a homogenized, pureed or freeze-dried powder form) is intended to provide production conditions which are microbiologically safe.

Raw meat intended for canning and unsterilized end-products were examined for the presence of spores of obligate anaerobic bacteria. Processing conditions ensuring industrial sterility for the canned meat have been worked out based on permissable numbers of bacteria and thermostability data.

Bacteriological testing of the freeze-dried powder prepared from blanched homogenised chicken meat was used to monitor possible sources of secondary contamination with enteritic bacteria, salmonella, staphylococcus and pathogenic clostridia after blanching. Permissable bacteriological counts for poultry meat baby foods have been worked out.

5:13

Antimicrobial activity and functionality of polyphosphates in reduced NaCl comminuted meat products

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Polyphosphates improve water holding capacity and may be useful in improving functionality of reduced NaCl products. The need for reduced NaCl meat products is due to the possible association of Na^+ consumption with hypertension. The preservative action of polyphosphates in meat products formulated with reduced NaCl is unclear. Objectives examined in this study included functional and antimicrobial properties of reduced NaCl comminuted meat products inoculated with Clostridium sporogenes spores in the presence of various polyphosphates.

Polyphosphates studied were sodium salts of acid pyrophosphate (SAPP), pyrophosphate (TSPP), tripolyphosphate (STPP), tetrametaphosphate (TTPP), hexametaphosphate (HMPP), and glassy hexametaphosphate (GHMPP). Two replicates with 5 kg batches per treatment were formulated with beef and pork (30% fat). The products also contained 16% added water, spices, nitrite and erythorbate. Each polyphosphate was formulated with 1.25% NaCl and levels of 1.25% and 2.5% NaCl were examined alone as controls. Polyphosphate levels tested ranged from 0.17% SAPP to 0.34% GHMPP. The sum of the ionic strength of each polyphosphate -1.25% NaCl treatment was equivalent to that of the 2.5% NaCl treatment. The comminuted mixtures were extruded into (303x406 and 208x108) cans, frankfurter casings and test tubes. Small cans were inoculated before thermal processing with heat-shocked (80°C, 15 min) spores of C. sporogenes PA 3679 (76/g) sealed and processed to 70°C, while slices from uninoculated large cans were inoculated after thermal processing and vacuum packaged. Inoculated cans and packages were stored at 27°C and gas production (swelling) was checked daily.

The increased emulsion losses of the reduced (1.25%) NaCl treatment were prevented by TTPP, TSPP, STPP and SAPP, while HMPP and GHMPP did not improve yield. Initial, cooked product pH of regular and reduced NaCl products were similar (6.21-6.26). SAPP reduced product pH (6.06-6.08), while the other polyphosphates increased pH (6.31-6.48). Gas and putrefaction were first observed in 3 days in cans with 1.25% NaCl and 1.25% NaCl-TTPP products. Swelling for SAPP and 2.5% NaCl treatments was first noted after 6 and 9 days, respectively. Packages with 1.25% NaCl alone and in combination with TSPP, STPP, TTPP showed first gas in 3 days, while first gas production in the 2.5% NaCl packages occurred in 6 days. In 5 days cans with reduced (1.25%) NaCl, and TSPP, STPP and TTPP showed >86% gas production, while cans with 2.5% NaCl, and SAPP, HMPP and GHMPP were <27% gassy.

In conclusion, with reduced NaCl (1.25%) product functionality, texture and antimicrobial activity were reduced; STPP, TSPP, TTPP and SAPP improved functionality of reduced NaCl products; HMPP and GHMPP showed little effect on functionality; and, antimicrobial activity of reduced NaCl products was improved only by SAPP and to some extent by HMPP and GHMMP.

5:14

Prevention of early spoilage of livers

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Livers and other offals are usually considered to be inherently prone to early spoilage, although there is no obvious reason why this should be so. In commercial practice livers are usually bulk-packed while warm. Thermocouples were inserted into commercial packs for temperature measurements. Initial temperatures usually exceeded 30°C and could approach 40°C. These temperatures were sometimes maintained for several hours, but even when effective cooling was applied soon after packaging 8 to 10 h were required for tub centres to reach chill temperatures (<5°C).

The effect of this apparent temperature abuse was determined with sheep livers individually packed in 650-ml plastic tubs with sealing lids. Tubbed livers placed in a 0°C chiller 1 h after their removal from the animal developed a flora of psychrotrophic lactobacilli. After 5 weeks the floras had not reached maximum numbers and the livers retained a fresh appearance. Livers subjected to temperature abuse by holding at 30°C for various periods before chilling developed floras dominated by Escherichia coli. At the abusive temperature, spoilage due to microbial activities had occurred by 24 h. Although overt microbial spoilage was not observed with shorter periods of abuse, liver texture and colour deteriorated after only 4 h at 30°C and subsequent development of the psychrotrophic spoilage flora was greatly accelerated. The short shelf life attributed to livers is evidently the result of bacterial growth at warm temperatures in the period following the removal of livers from carcasses. Immediate, effective cooling is therefore necessary to prevent both early spoilage of livers and the significant health hazard posed by proliferation of enteric bacteria. For regulatory and quality-control purposes, some convenient method of estimating bacterial proliferation during liver cooling is required. Direct determination is time consuming and may not be practicable in commercial circumstances but, in principle, any increase in bacterial numbers can be estimated from the temperature history of livers and appropriate constants for the organism of greatest concern, E. coli. Constants were determined from √growth rate vs temperature curves for 6 strains of E. coli isolated from livers and grown under anaerobic conditions on a rich medium. Calculated proliferation of E. coli was compared with that determined directly by plating of drip samples onto Violet Red Bile agar at the initiation and conclusion of various cooling regimes. Calculated increases were closer to average values from replicated cooling regimes than the extreme values for any particular regime. Estimation of microbial growth therefore seems to offer an appropriate basis for evaluating offal cooling processes.

5:15

Discolouration of cured meat pigment by bacteria

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Discolouration of the cured meat pigment - either by chemical or bacterial agents - has been known for many years. However, due to better knowledge of the possible causes and more efficient means of prevention, much less has been reported on the topic recently. Yet there are certain circumstances where because of the coincidence of chemical, physical and bacterial factors, discoloration of the cured meat pigment can still take place. In our experiments, the bacterial factor has been examined in a case which occurred quite frequently during the summer months.

Bacteria were isolated from emulsion type of meat products which showed discoloration, and tested for discoloring ability. Selective media for lactobacilli and an MnO_2-medium were used. On the MnO_2-medium, catalase-negative and H_2O_2-producing bacterial form a halo around the colonies as the MnO_2 is reduced to a colorless manganese compound. The MnO_2-medium was prepared by suspending finely ground MnO_2 (5%) in a 10% gelatine solution to prevent sedimentation. This suspension was sterilized, 6% added to molten plate count agar when hot, stirred and poured into Petri-dishes.

The bacteria forming a halo on the plates were isolated, biochemically tested, minimum and maximum growth temperature, as well as heat resistance, determined. Minimum growth temperature proved to be 6°C, where colonies were formed in 10 days. No growth occurred above 45°C. Heat resistance was tested by inoculating a known number of pure cultures into a heating medium, the temperature of which was adjusted and held by an ultrathermostat. Since the volume of heating medium was 50 times higher than that of the inoculum, this latter reached the desired temperature without delay. After certain time intervals samples were taken and the count of the survivors determined by serial dilution and plating. Using nutrient broth as heating medium, a D_{10}-value of about 2 mins was found at 65°C. Keeping in mind that in meat emulsions heat resistance is always higher, it is understandable that these bacteria may survive regular heat treatment of meat products and may thus cause discoloration. Survival is further supported by the fact that, because of low minimum growth temperature, growth occurs even at chilling temperatures, thus increasing the initial count. According to preliminary identification studies, the strain isolated is an atypical Lactobacillus, resembling L. leichmannii.

5:16

Nutritional requirements of lactobacilli spp. from meat

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Lactobacilli isolated from fresh meat are often called "atypical streptobacteria". They belong to the Streptobacterium group but differ from typical strains in their failure to ferment many sugars and in their ability to degrade arginine at low sugar concentrations. Although nutritional behaviour may be useful to identify and classify lactobacilli no information about this is available for these atypical strains.

The amino acid and vitamin requirements of L. curvatus, L. sake and some unidentified strains commonly found on fresh meat have been studied and compared with L. plantarum or L. casei. For this study the basal medium of Ledesma et al. (J. appl. Bact. 42, 123-133 (1977)) was used with some modifications, the concentration of following amino acids, proline, glycine, arginine was increased.

Vitamin requirements of atypical streptobacteria were found to be the same as those of L. plantarum or L. casei; niacin and calcium pantothenate were essential for all strains, and some strains also required pyridoxal phosphate and riboflavin.

Strains isolated from meat differed from typical streptobacteria in their greater requirement for specific amino acids; aspartic acid, glycine, arginine, histidine and proline are often essential for their growth. Lactobacillus curvatus appeared to constitute a sharply defined species within the Streptobacterium group: this was the most fastidious species and required all amino acids except alanine and tryptophane. Strains related to L. sake also exhibited a specific nutritional behaviour: only four amino acids (phenylalanine, threonine, lysine, tyrosine) were found to be non-essential. Some strains closely related to L. sake had, however, a different amino acid requirement: cysteine and serine were not required but threonine and lysine were found to be essential; these strains may constitute a new species.

In conclusion it may be pointed out that the inability of Lactobacillus strains from meat to synthesize many amino acids is not surprising since their natural environment is rich in these substances.

5:17

Time course of volatile compound formation during microbial growth on beef stored at + 5°C in air

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Volatile end products of microbial growth on meat stored refrigerated in air are major components of 'off odours' which signal the end of shelf-life. Inoculation of sterile meat with pure cultures has enabled many of these compounds to be identified. In this study the time course of changes in the relative amounts of various volatile compounds in a natural, mixed flora spoilage situation was followed. Stewing or braising steak from local butchers' shops was stored aerobically at + 5°C and total viable counts (TVC), and counts on selective media for Pseudomonas spp., Brochothrix thermosphacta and Enterobacteriaceae were made daily. At the same sampling times the odours of the meat were assessed and the composition of the headspace volatiles analysed using a porous polymer entrainment technique followed by combined gas chromatography-mass spectrometry.

Total microbial numbers at purchase were between 10^5 and 10^7/g and reached c. 10^9/g after 3 days. During this period amounts of acetoin, diacetyl, 2-methyl propanol and 3-methyl-1-butanol increased and the raw meat developed a 'non-fresh' odour. When microbial numbers exceeded 10^9/g ethyl esters of a range of C_2-C_6 acids were detected which subsequently increased in concentration during extended storage, the odour of the meat now being described as "sweet" or "fruity". Sulphur containing compounds were not detected until the TVC exceeded 5×10^9/g by which time the meat showed obvious visual signs of spoilage and possessed a sweet putrid odour.

These results with a natural mixed flora are in agreement with pure culture studies regarding the identities of volatile spoilage compounds, and further indicate that there could be a consistent pattern of change in the time course of their formation. In addition, many of the volatile compounds detected during the early stages of storage are normally associated with the growth of Br. thermosphacta. This observation is consistent with the presence of substantial numbers of this organism on our samples.

5:18

A study of the delay in the appearance of swelling in under-processed canned meats

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Bacteriological swelling in under-processed canned meats was studied over a period of 22 years (1962-1983) in a large French preserves factory. During this period 857 x 10^6 cans were manufactured including all the usual types. Systematic incubation (10 days at 35° ± 2°C) sorted lots into those without problems, those with non-bacteriological swelling, and those with swelling caused by bacterial growth. Amongst the latter category, laboratory examination permitted distinction of preserves swollen because they were recontaminated after heat-processing (particularly during cooling) from those swollen because they were under-processed.

The under-processed category was composed of 99 lots containing 199,484 cans which constituted 0.023% of the total manufactured during the 22 years. The incidence of swelling was variable: from 234-266 cans for 30 lots, from 314 to 675 for 14 lots, and from 1,008 to 6,220 for 55 lots (including 50 with 2,038 to 4,665). A lot was defined as a collection of cans of the same product and size produced on the same day and including swollen cans resulting from under-processing. Swollen cans sometimes came from only one autoclave (250 cans) but most often originated from several.

There were three different types of under-processed lots:

1. In 7 lots out of 99 (7.1%) swelling was relatively rapid. All cans were swollen between 10 days and 3 months. Six of these lots included swollen cans from only one autoclave.
2. In 12 lots (12.01%) swelling was quite rapid (10 days to 6 months) but only affected 30-40% of the cans. No swelling was observed later than 6 months.
3. In 80 lots (80.81%) swelling was common at 10 days and 1 month and then either continued at the same high level or decreased regularly with time until the incidence became very low at each examination (in some cases nil at the final time).

In conclusion, the latter type demonstrated that in some cases of under-processing swelling delay was extremely long. It is confirmed that bacteriological control is inadequate for testing a commercial lot of preserves, and that the best method to assure safety in these products is to follow Good Manufacturing Practice (GMP). One under-processed lot in which rapid swelling occurred contained a total of only 0.02% swollen cans after 3 years incubation, which corresponds to the usual incidence in lots which have been processed normally.

5:19

Application of Direct Epifluorescent Filter Technique as a Rapid Method in Microbiological Quality Assurance in the Meat Industry.

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In recent years rapid methods in microbiological quality assurance (MQA) have aroused considerable interest in the meat industry. The reasons for this are the advantages related to the speedy release of products, and that rapid results in monitoring critical control points offer the possibility for early corrections during production. Furthermore, rapid results enable the manufacturer to select raw materials according to their microbiological quality.

In Denmark none of the rapid methods, e.g. ATP, impedance, radiometry, microcalorimetry and direct epifluorescent filter technique (DEFT), has been used so far in the meat industry for these purposes.

The object of this study has been to adapt the direct epifluorescent filter technique (DEFT) to MQA in the meat industry. In the course of the work, attempts have been made to select well-defined areas where it was believed that DEFT could be used with advantage. DEFT-results are obtainable within one hour.

Both raw and heat-treated meat products were examined. The most promising results were obtained with raw ground beef, where good agreement was found between DEFT and the standard plate count (SPC). All these products had bacterial counts of 10^5/g and above, and the correlation coefficient was 0,79. For heat-treated meat products, good agreement between DEFT and SPC was found for products with high counts at the end of shelf life (10^8/g), whereas very poor agreement was found in products with low counts. Nevertheless, useful information with DEFT was also found in these cases, since it was possible to estimate approximately the number of bacteria present before heat-treatment. The study clearly showed that DEFT could not distinguish between live and dead cells, and also that this was not a prerequisite for using DEFT.

It was concluded that DEFT could be used with advantage in a MQA-programme for ground beef to check that a microbiological specification of 10^6/g was adhered to. However, automatic counting could not be used for this level. This limits the capacity to 20 samples per person per day. It is hoped that further development of the DEFT system will overcome this problem.

5:20

Influence of electrical stimulation on distribution and rate of migration of sodium nitrite, sodium chloride and glucose in pork tissue

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The objectives of this study were to evaluate the effect of electrical stimulation of pork carcasses on the distribution and migration rate of curing ingredients.

One side (left) of each carcass was stimulated 45 min. post slaughter (50 electrical impulses of 400 V alternating current of 1.5 sec each in duration followed by 1.5 sec of no current) and the other side (right) was used as a control (non-stimulated). The triceps brachii muscles were removed 24 hr post-mortem from both sides of the chilled carcass. Cylindrical samples of 1.5 cm in diameter and 5 cm in length (parallel to the muscle fibers) were prepared from the muscles. These cylindrical samples were tightly placed into plastic tubes in an effort to prevent migration of curing solution between the sample and sides of the tube.

Two and one-half ml of a curing solution, composed of 20% NaCl, 6% glucose and 0.16% $NaNO_2$, was added to the tubes above the sample. Both stimulated and non-stimulated samples in the tubes were held at 3°C - 5°C and sampled at 24, 48 and 72 hrs. At each sampling time, the excess cure above the sample was discarded and the cylindrical sample was removed from the tube and divided into 4 cylindrical segment levels of 1.25 cm in length from top (adjacent to curing solution) to the bottom of the sample. These segmented samples were each individually analyzed for $NaNO_2$, NaCl and glucose. Concentrations of all curing ingredients at each sample level increased in a linear ($P<0.01$) fashion with time. Electrical stimulation resulted in significantly higher levels of $NaNO_2$, NaCl and glucose in each 1.5 cm meat segment of the samples when compared to equivalent tissue levels of non-stimulated samples at each sampling time of 24, 48 and 72 hrs.

The analysis of variance indicated that electrical stimulation caused significant ($P<0.01$) improvement in cure absorption and migration of curing ingredients ($NaNO_2$, NaCl, glucose) into all layers of the meat cylinder.

5:21

Changes in proteins and fats of old hens meat as influenced by pre-freezing treatments

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The effect of some pre-freezing treatments on some tissue changes of old hens during frozen storage was studied. Highest total volatile nitrogen (TVN) and total bacterial counts (TC) were found in frozen breaks, followed by leg and wing base; trimethylamine nitrogen (TMAN) and amino nitrogen (AN) were highest for the leg followed by breast and wing base. Post-rigor freezing enhanced the breakdown of proteins, while pyrophosphate treated samples had lowest TVN, TMAN, AN and TC. Similarly pyrophosphate treatment led to a reduction in lipid oxidation as indicated by the thiobarbituric acid value (TBA), but propolis antioxidant was more efficient in this matter. Post-rigor frozen samples were subject to greater lipid oxidation than pre-rigor frozen samples. TBA value was highest for leg followed by breast and wing base at any given time of frozen storage. Fatty acid composition changes confirmed the results of TBA value, where propolis treated samples were more stable against lipid oxidation, followed by pyrophosphate and citric + ascorbic acid samples as indicated by the rate of total saturated fatty acid increase and total unsaturated fatty acid decrease. During frozen storage apparent increase in total saturated fatty acids was mainly due to the increase in C_{16} and C_{18} acids, while the decrease in total unsaturated acids was mainly due to reduction in $C_{18:1}$ and $C_{18:2}$.

5:22

Limiting conditions for yeast growth on frozen meat

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Yeasts are not usually thought to play any significant role in meat spoilage as faster growing bacteria cause spoilage at chiller temperatures while spoilage of frozen meats is ascribed to moulds. Bacterial spoilage of chilled meats is undoubtedly predominant but recent research on spoilage moulds has shown that these moderately xerotolerant psychrotrophs usually develop on unfrozen meat when surface drying precludes bacterial proliferation. It is therefore possible that in some circumstances psychrotrophic yeasts may be significant spoilage organisms of frozen meats.

When lamb loins wrapped in gas permeable plastic film were held at -5°C they developed a yeast microflora which reached maximum numbers of $10^6/cm^2$ and appeared as discrete pin-head colonies after 20 weeks storage. Visible mould colonies did not appear until after 35 weeks storage. Four species contributed to the yeast flora; Cryptococcus laurentii var laurentii, Cryptococcus infirmo-miniatus, Trichosporon pullulans and Candida zeylanoides. Cr. laurentii predominated at all stages of flora development. Numbers of yeasts initially present on lamb were low but showed seasonal variation. In winter Cryptococcus and Candida species predominated at 10-50 cells/cm^2. Numbers of these species were similar in summer but total yeast numbers were greater by an order of magnitude because of the predominance of Rhodotroula species, in particular R. glutinis.

The limiting conditions of temperature and a_w for growth of these yeasts were examined. Observed a_w-minima for growth at 20°C on nutrient agars with a_w adjusted by the addition of glycerol were R. glutinis, 0.94; Cr. laurentii and Cr. infirmo-miniatus, 0.92; and C. zeylanoides and T. pullulans, 0.90. Minimum growth temperatures on media containing glycerol just sufficient to prevent freezing were R. glutinis, -3°C; C. zeylanoides, -5°C; and other species -6.5°C. The two Cryptococcus species grew comparatively rapidly at -5°C after a lag of 4 weeks with generation times approximating 10 days.

It can be concluded that yeast spoilage is likely to develop on meat held at marginal freezing temperatures of -6.5°C and above. The dominance of Cr. laurentii is not due to any specific growth rate advantage under these conditions but is a function of the initial flora composition and distribution. The occurrence of yeast species in frozen meat spoilage floras would seem to offer a sensitive diagnosis of suspect temperature abuse during storage of frozen meats.

Influence of freezing on the morphology and survival of psychrotrophic bacteria in meat

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The influence of freezing at -18° and -196°C on the morphology of the psychrotrophic bacterium Pseudomonas fluorescens, isolated from chilled meat, was studied. A 24 hour bacteria culture in a broth was frozen in air at -18°C for one hour (slow freezing) and in liquid nitrogen for 90 sec. (quick freezing). Two defrosting regimes were used corresponding to each freezing regime: quick, 5 min. at 30°C, and slow, one hour at 18°C. During preparation of the samples for electron microscopy (e.m.), fixation of the bacteria in formalin vapour and a drop dialysis were used. A method of metal tinting on a spraying plant (Hitachi 4) was used to increase the contrast of the intact cells. Platinum alloy was used as a tinter. The morphological investigation of the bacteria was carried out on an UEMB-100 B electron microscope at an accelerating voltage of 75 kV and aperture diaphragm of 35 μm. Sample sections were 200-300 A thick.

The e.m. investigations showed destructive changes in Pseudomonas fluorescens cells, depending upon the methods of freezing and defrosting. During quick defrosting of quick-frozen cultures, the cells lost their typical rod-type form and developed large conglomerates on the surface; during defrosting of slow-frozen cultures, the cells took incorrect, diffused forms. During slow defrosting of quick-frozen cultures much disintegration of cells was observed; slow-frozen bacteria cells exhibited clear detachment of the cell wall from the protoplast.