8:1

SESSION 8: ANALYTICAL CHEMISTRY

The differentiation of meat species by Direct Probe Mass Spectrometry

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The application of a novel technique for the differentiation of meat species, direct probe mass spectrometry (DPMS), is described.
Three experiments were performed in which samples of freeze-dried aqueous extracts of meat from different species were subjected to replicate analysis by DPMS. In the first study samples were obtained from six different muscles from each of three animal species, cattle, chicken and rabbit. In the second study one sample was taken from each of four individual animals from each of six species, cattle, chicken, horse, pig, rabbit and sheep. In the third study three species, beef, horse and sheep were examined in more detail using samples from six individual animals from each species.
DPMS is a low temperature pyrolysis technique (max 350°C) in which the sample is thermally degraded adjacent to the ion source of the mass spectrometer. Continuous scanning of the instrument produces a series of spectra across the evolution profile which are then averaged to provide a single fingerprint of the sample. The averaged spectra were analysed by statistical procedures which determined the ions responsible for the greatest degree of discrimination between the species, and the intersample relationships were displayed either as a two-dimensional scatter diagram or non-linear map.
The results showed that in all three experiments DPMS was able to differentiate the species of origin of these meats despite the closeness of the beef, horse, and sheepmeat groups which was revealed in experiment 2. Experiment 1 also demonstrated that there is greater inter- than intra- species variation, an important consideration when dealing with samples of unknown origin. The reliability of the analysis procedures was tested with further replicates, not included in the original data analysis, which were treated as unknowns. These test samples fitted extremely well into their respective groups indicating that no undue distortion had been induced by the discriminant analysis techniques.

8:2

Direct probe mass spectrometry and the classification of lactic acid bacteria from vacuum packed meats

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The microbial flora on stored vacuum packed meats is usually dominated by lactic acid bacteria which grow to c $10^8/cm^2$ and probably affect shelf life. It is desirable to understand the nature of these bacteria. This paper describes their classification by direct probe mass spectrometry (DPMS) which thermally degrades whole bacterial cells adjacent to the ion source of a mass spectrometer, producing a spectrum characteristic of the sample. Strains are grouped according to similarities between their spectra.

Forty strains of lactic acid bacteria from vacuum packed beef, pork, lamb and bacon were studied. Each strain was analysed in triplicate using 50 µg of cells from a plate culture incubated for 48h at 25°C. Analyses were performed on a Finnigan 4000 quadrupole mass spectrometer in which the probe containing the cells was temperature programmed at 60°C min^{-1} from ambient to 300°C. Each spectrum consisted of intensity values of 300-400 ions which were normalized to the total ion count to remove variations due to sample quantity. Relationships between the spectra of different strains were determined by multivariate statistical techniques using sets of ions selected for reproducibility and strain discrimination.

Five groups of strains were distinguished which corresponded closely to the groupings of strains detected in a previous numerical taxonomic study using traditional morphological, physiological and biochemical tests. Two groups contained all 12 representatives of a cluster of unidentifiable non-aciduric streptobacteria whose sub-division is supported by other taxonomic evidence. All twenty-one strains from a cluster of aciduric streptobacteria provisionally identified with L. sake were contained in two further groups. The sub-division of these aciduric strains has yet to be verified by other techniques. The fifth group contained Leuconostoc strains.

The technique of DPMS has both clarified and deepened the classification of lactic acid bacteria on vacuum packed meats and has facilitated the selection of representative strains for use in pure culture studies to determine the roles of the various groups in spoilage.

8:3

The influence of multi-extraction on the level of the residual nitrite determined in model, scalded, comminuted meat products.

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Attempts have been made to evaluate the influence of multi-extraction on the amount of free-nitrite determined in model, comminuted, scalded sausage-type products, processed from either beef or pork. After grinding through a plate with a hole diameter of 2 mm, beef or pork was cured during homogenization by adding 200 ppm nitrite + 200 ppm sodium ascorbate, both dissolved in water, in an amount equivalent to 30 per cent of the meat. Experimental sausage mince was placed in a 150 ml glass beaker and after 2 h of curing at 4°C, scalded in an 85°C water bath until 80°C was reached in the geometric centre, and then cooled in water for 5 min.

Residual nitrite was determined 24 and 72 h after completion of the processing. Two 50 g samples of a reground model product were extracted with 25 ml of saturated borax plus 75 ml of distilled water in a boiling water bath for 30 min with occasional mixing. After deproteinizing and centrifuging, free nitrite was determined in the extracts using Griess reagents. The residue, after first extraction, was re-extracted 4 times i.e. 5 times in total. It was observed that only 50 per cent of the total amount of free nitrite determined during 5 consecutive extractions could be determined after the first extraction, and 75 per cent after two repeated extractions; the fifth extraction did not significantly influence the total quantity of the residual nitrite. It was also found that the nitrite was more loosely bound with beef than with prok.

It was concluded that the present practice of residual nitrite determination in cured meat products, based on one extraction only, does not allow determination of the real amount of free nitrite in commercial cured meat commodities, and therefore appropriate amendments to the present analytical procedure should be urgently considered.

8:4

Residue levels in cows after oral ingestion of methylthiouracil

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Regulations in the EEC prohibit the use of methylthiouracil (MTU = 4(6)-methyl-2-thiouracil) in cattle fattening; residues of this potent antithyroid drug in meat may constitute a health hazard. Due to the lack of a sensitive determination method, there is little information on the residue levels of MTU in meat, plasma or excreta after administration of this goitrogen to cows.

We have studied the elimination of this drug in different tissues, plasma and excreta after oral administration of MTU to cows. The residues of this goitrogen were quantitatively determined at the ppb-level by a specific HPTLC-fluorescence method. Experiments were performed on 2 groups of cows. In the first group, the elimination of MTU was studied in plasma, milk, urine, thyroid and muscle after a single oral administration of 5 g MTU (8 cows). In the second group the disappearance of MTU in tissues and body fluids was determined after ingestion of a daily dose of 5 g MTU during 2 weeks (2 cows), 3 weeks (3 cows) or 4 weeks (3 cows). In the first group, there was a rapid parallel decline in the MTU levels of urine, plasma and milk up to 80 hr after treatment ($t_{\frac{1}{2}}$ = 6 hr). In the thyroid the MTU concentration was always higher and the residue level decreased in function of the period after ingestion (2-18 days) with an apparent half-life of 3 days. In the second experiment, at least two phases were observed in the disappearance of MTU. After withdrawal, the MTU levels of plasma and urine showed a very rapid decline ($t_{\frac{1}{2}}$ = 14 hr) during the first 4-5 days. Then the elimination rate decreased considerably ($t_{\frac{1}{2}}$ = 21-24 days) so that, after a withdrawal period of more than 40 days still distinct levels ($\geq$ 10 ppb) were found in urine or plasma. A parallel decrease in MTU concentration was observed in thryoid and muscle (M. diaphragma) during a withdrawal period of 8-80 days ($t_{\frac{1}{2}}$ = 9 days). The residue levels during that period were 5 times higher in urine than in plasma; MTU-concentrations in the thyroid were 3 times higher than in meat.

For regulatory control on living animals urine sampling is preferred. During control in the slaughterhouse thyroid tissue should be selected as a target tissue for MTU detection.

8:5

Determination of methylthiouracil and analogous thyreostatic drugs

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Treatment of cattle with thyreostatic drugs is detected by the residues present in plasma, excreta, meat or organs of the animals. Optimal detection of illegal treatment with thyreostatics will be achieved through selection of the tissue or physiological fluid with the highest residue concentration and the use of a reliable, sensitive detection method. A specific detection procedure for thioracil and analogous compounds, based on fluorescence induction of the NBD-derivatives (7-chloro-4-nitrobenzo-2-oxa-1,3-diazole) with cysteine, has been described previously. However, the clean-up procedure for the extracts did not allow full exploitation of the sensitivity of this reaction. A rapid and selective extraction procedure for thyreostatic drugs is presented here allowing quantitative determination of the thiouracils in various extracts of biological origin.

Homogenates of thyroid, organs, meat or milk, urine and plasma are extracted with methanol. Clean-up of the extracts is performed through adsorption on a mercurated resin. After desorption, the thyreostatics are derivatized with NBD-Cl, extracted, and separated by thin-layer chromatography (HPTLC). Fluorescence is induced by spraying with cysteine and the relative fluorescence measured against an internal standard (4(5,6)-dimethyl-2-thioracil). Extraction yields of the various thioracils added to extracts at levels of 10-100 ppb vary between 60-80%. The method has been routinely applied to samples taken from cows, slaughtered after illegal treatment with methylthiouracil (MTU). The residue distribution was studied amongst different tissues taken from the same carcass. Highest residue levels of MTU were observed in the thyroid (30-50 ppm) with concentrations 20-100 times higher than in different muscles of the corresponding carcasses. Cooking experiments demonstrated that MTU-residues in meat are not appreciably destroyed by prolonged heating.

This single step extraction procedure results in an increased selectivity and allows quantitative recovery of the thyreostatics from biological extracts. It speeds up the analysis while improving resolution of the chromatography. This procedure surpasses existing methodology in speed, simplicity and sensitivity of detection of thyreostatic drugs in extracts of biological origin.

8:6

Amino acid composition and N^{τ}-methylhistidine content in the protein analysis of meat products.

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In general, it is difficult to comply with requests for the analysis of specific proteins in cooked meat products. Analyses including the preparation of a protein extract are hampered by a lowered solubility of the proteins, so that other approaches have to be used, especially for quantitative analyses. The approach based on the amino acid composition and a multivariate analysis revealed useful data for some products, but yielded too many possible compositions for other products.
In this study the applicability of a combination of general amino acid composition, specific amino acids (e.g. hydroxyproline) and N^{τ}-methylhistidine content has been tested for the determination of lean muscle meat content and other protein-containing components in extended meat products. For this purpose the N^{τ}-methylhistidine and specific amino acid contents of different muscles, organs and other raw materials for the manufacture of meat products should meet certain conditions.
The variations of N^{τ}-methylhistidine in various muscles and organs were studied, using a HPLC technique for the assay. It was found that the methylhistidine content of various muscles of different animal species varied only moderately. It amounted to an average value of 69.5 ± 6.9 mg methylhistidine per 100 grams of connective tissue-free protein, except for heart muscle (pig 31.6 mg/100g). The methylhistidine content of different organs was sometimes substantial (tripe, spleen, tongue, rind), but showed low values for kidney, liver, brain, udder and blood.
When considering the raw materials mostly used in the manufacture of meat products, the methylhistidine content seems to be a valuable factor in analyzing meat products by the multivariate analysis. This indication was confirmed very clearly with some self-made meat products containing lean muscle meat, tendons, liver, soya, casein, wheat gluten and potato protein. Inclusion of this content in the amino acid composition of raw materials and meat products yielded highly probable compositions which agreed very well with the real compositions.
This improvement in the analysis of proteins in extended meat products will subsequently be checked with industrial products.
Apart from the use of N^{τ}-methylhistidine for calculation of the protein composition, an index based on the methylhistidine content would be a useful measure for the lean meat content of meat products and might thus be used as a quality index.

8:7

Anabolic treatment and quality of meat

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Public health officers and consumer organizations often complain about the harmful influence of anabolics on the quality of meat from treated animals. These complaints involve the meat quality itself (tenderness, water holding capacity, colour) and the possibility of residues of anabolics in meat that are regarded as posing a potential health risk.

We have evaluated the influence of implantation of trenbolone (Torelor^{R}) on the quality of meat from young bulls. The composition of Longissimus dorsi muscles was similar in anabolised and untreated animals in terms of total protein content and amino acids after hydrolysis. Comparison of protein gel electrophoresis patterns of sarcoplasmic and myofibrillar fractions did not reveal any differences between treated and untreated bulls. Muscles from both groups of animals were found to contain the same amount of total pigment. Results from total collagen determination, performed on the Diaphragma, showed significantly lower values for anabolised bulls. The assay of "hydroxyproline containing material" solubilised in the cooking juice of muscles was found to be a good indicator of their tenderness, which was also determined by Warner-Bratzler shear force values. It appeared that the anabolisation of bulls did not modify the tenderness nor water holding capacity during cooking.

The amount of trenbolone residues in muscle, extractable with organic solvents, was measured by radio-immuno assay (6,7-[^{3}H]-β-trenbolone, anti-17β-trenbolone hemisuccinate bovine serum albumin antiserum), the assay sensitivity being less than 0.01 ppb. Residues were found to be very low: less than 0.15 ppb at day 66 after implantation. These values progressively decreased to 0.02 ppb at day 280 post-implantation. Nevertheless, previous data (Ryan and Hoffmann, 1978) suggest that bound residues (not extractable with organic solvents represent 90% of total trenbolone residues in meat or liver of treated animals. The formation of bound residues of trenbolone was studied in vitro using bovine liver microsomes. This study showed that hepatic monoxygenases can generate reactive trenbolone metabolites, which are able to bind to microsomal proteins or can be detoxified by conjugation to glutathione or glutathione-S-transferases. Taking into account the physico-chemical properties (water solubility, chemical stability) of compounds involving covalently bound residues, bound residues of trenbolone are most probably not toxic to human consumers.

Ryan, J.J. and Hoffmann, B. J.A.O.A.C., 1978, 61, 1274.

8:

8:8

Possibilities for connective tissue-free muscle protein determination in cooked sausages by a dye-binding method

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Connective tissue-free muscle protein(CTFMP) determination by subtracting the content of connective tissue from total protein content is a laborious and time-consuming procedure. A simple dye-binding method for a direct CTFMP determination in meat-only sausages is proposed.

Aliquots of the suspension of cooked sausages in saturated citric acid solution were allowed to react with a standardized solution of Amido Black 10B; the remaining dye concentration was measured colorimetrically; thus the estimated dye loss could be related with protein content.

The mean estimated dye-binding capacity(DBC) values,expressed as mg bound dye(Amidoschwaz 10B, Merck, a_{620} = 73.3 cm^{-1} (g/l)$^{-1}$) per mg protein (N x 6.25), of the principal nitrogen-containing fractions of the muscle (myofibrillar, sarcoplasmic, and stroma proteins, and nonprotein nitrogenous substances) were 0.57, 0.44, 0.06,and 0.0, respectively. Heat treatment at 80°C for 90 min did not change the DBC's of the myofibrillar and sarcoplasmic protein fractions, while about a 2-fold increase was observed when the stroma fraction had been treated under the same conditions.

The DBC values for various cooked sausages range from 0.325 to 0.440 (0.398 ± 0.030, mean ± s.d.)being strongly dependent on their connective tissue content. While the connective tissue nitrogen comprises 8 to 23% of the total nitrogen content, it contributes to only as little as 2% of the DBC of the whole sausage homogenate. Myofibrillar and sarcoplasmic proteins account for the remaining 98%. Therefore, the quantity of dye lost when the suspended proteins react with Amido Black 10B under the specified conditions, could be used as a direct measure of the CTFMP content provided a suitable conversion factor or reference protein(s) be chosen.

The usage of a single numerical factor for the direct conversion of the dye bound into percent CTFMP content is a promising but still inapplicable approach since the various commercial batches of dye differ greatly in their purity and dye content. With the connective tissue N not taken into account, the corrected DBC value averages 0.473 (± ·020) mg bound dye per mg protein (N x 6.25), which is close to the DBC value of BSA(Fluka) - 0.480 . Thus the latter could be used as a reference protein to calculate % CTFMP content from the A_{620} values measured.

8:9

The basic method of foreign matter residues monitoring in meat and foodstuffs of animal origin

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According to experimental experience a basic method for the monitoring of residues of foreign matter in foodstuffs of animal origin has been evolved. Monitoring is being organized within the framework of the Veterinary public health service and provides information for all processing plants. Samples of raw materials and foodstuffs are taken by the Veterinary public health service in all production plants in the region. Apart from other testing, the samples are analyzed for the determination of residues of chlorinated pesticides - HCB, lindane, aldrin, DDE, DDD and DDT isomers as well as residues of PCBs by multidetection methods. Chlorinated pesticides are extracted from the sample together with fat using petrolether. Fat is eliminated from extracts by use of a florisil column (Florisil 60/100 mesh, column 30cm x 1.5 cm i.d.). 8% diethylether in petrolether is used for the elution of pesticides from the florisil column. The pesticide eluate is concentrated in a rotary vacuum evaporator to a volume of 2ml. 1 to 5 µl of sample is injected into the column of a gas chromatograph, according to the pesticide content. A glass column 2m x 3mm i.d. packed with 1.5% OV-17 + 1.95% OV-210 on Chromosorb W HP 100/120mesh is used. The isothermal analysis takes place under the following conditions: 230°C injection port, 205°C column and 300°C detector (ECD, Ni-63); carrier gas flow (N_2) through the column 20-40ml/min, through the detector 70-90ml/min. The results of the sample analysis are compared with analysis of standard solutions of pesticides. The effectiveness of the described method is 98-102% in the case of HCB and 92-96% in the case of ppDDT. HCB, lindane, aldrin, DDE, DDD and DDT isomers can also be determined in the presence of PCBs with the use of multidetection methods. The method is suitable for the analysis of samples with a low chlorinated pesticide residue content (0.01-0.2 mg HCB/kg of fat, 0.02-0.4 mg lindane/kg of fat, 0.05-1mg DDE and 0.1-2mg DDT/kg of fat). In cases where the content of pesticide residues is higher, the method can be used after the fluorosil clean-up stage. The results of all analyses (1200-1500 per annum) are registered according to the sample type, the raw material processor, up to the level of the primary agricultural producer and his locality. This multidetection method will enable us to take effective measures in the food processing plants as well as with the primary agricultural producers wherever cases of increased residue levels are detected.

8:10

Meat analysis by infra-red transmission

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Infra-red transmission (IRT) has been applied to the determination of fat, protein, carbohydrate and moisture in a variety of meat and fish products. The Superscan instrument (Foss Electric) uses measuring and reference wavelengths in the range of 4-10µm. The equipment consists of a reactor, pump, measuring unit and printer or micro-computer. The analytical procedure consists of representative sampling, digestion of an 11g sample with 100ml dispersing reagent in the reactor and measurement of the digest. A complete analysis may be carried out in 10 minutes.

Accuracy obtainable against standard methods is heavily dependent upon the quality of sampling, but standard deviations of better than ±0.5% for fat, protein and carbohydrate and better than ±1% for moisture are obtainable. Successful applications have included raw meats, poultry, bacon, cooked ham, fish, pet food, burgers, spreads, pastes and pates, pie fillings, pastry, fish fingers and sausages. Depending upon the product being manufactured, the Superscan may be applied to raw materials and/or intermediates and/or finished products.

It is generally found that different types of meat (e.g. beef, pork and lamb) may be analysed using the same calibration. Similarly a number of types of sausages or pie filling etc. may be grouped together.

A micro-computer is normally interfaced with the measuring unit. This allows any calibration to be applied at the touch of a key. It also allows the weighing of sample and diluent to be carried out fairly roughly, with the computer applying corrections to the results for dilution ratio. In the U.K. it is possible to obtain the meat content of the product directly on the computer printout. This is based upon the traditional Stubbs-More formula, obtaining meat content from the results for fat, protein and carbohydrate.

For some samples, the highly efficient, dual cutting-edge Mohle Boy grinder/mincer is employed, particularly to eliminate long fibres.

Compared with the near infra-red reflectance technique, IRT requires more sample treatment, but is much more reliable.

8:11

Relationship between boar taint and skatole determined with a new analysis method

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With the aim to establish an objective and rapid method for the identification of young boar carcasses with taint, a new colourimetic method for the determination of skatole in extracts from adipose tissue has been developed.

The method is able to detect concentrations down to 0.02-0.04 ppm skatole in back-fat; recovery is measured to 95-105%, and the whole procedure including homogenisation and extraction can be carried out within 10 minutes by using continous flow analysis equipment.

The method has been tested on 201 entire male pigs of around 90 kg live weight at slaughter, selected to give a high incidence of boar taint. The analysis results have been compared with organoleptic taint evaluations carried out by a trained panel. 60 of the samples were also assessed by a standard taste panel.

The correlation between taint scores and the analysis results (reported as skatole equivalents) was 0.73 ($p<0.001$), which was almost as high as the correlation between the two independent panels. Based on a preliminary acceptability limit, 97% of the samples were correctly classified as acceptable/non-acceptable by the chemical method, and only one sample was seriously misclassified.

It is concluded that the method seems to be well suited as a quality ensurance test for entire male pig carcasses.

8:12

Boar taint and bitter taste as affected by androstenone and skatole

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Boar taint intensity and the concentration of androstenone and skatole were investigated in samples of backfat from 143 boars slaughtered at 110 kg live weight. Androstenone was determined with a radioimmuno assay and skatole with a new rapid Danish method. In addition, meat samples from the loin were evaluated by a taste panel for boar taint, tenderness, overall taste and bitter taste.

Skatole was quite strongly correlated with boar taint in fat (r=0.69), as well as with the meat scorings for taint, overall taste and bitter taste (r=0.67; 0.57 and 0.49, respectively). A lower correlation was obtained between androstenone and boar taint in fat (r=0.46) and especially between androstenone and the meat variables (r=0.23, 0.34 and 0.29, respectively). The correlation between skatole and androstenone was 0.31. Many boars classified as obviously or strongly tainted in fat were also identified by the taste panel when scoring the meat.

It is concluded, that androstenone alone is a reasonably good predictor of boar taint presence in backfat. As androstenone is fat-soluble but not water-soluble, this substance does not determine the overall taste or bitter taste in meat from boars to any significant degree (R^2=11.3 and 8.3%, respectively). Skatole is both fat- and water-soluble, and would thus appear to be a better determinant not only for boar taint in fat, but also for the overall taste and bitter taste in meat (R^2=33.0 and 24.4%, respectively).

The skatole assay is quickly done, and is therefore suitable for use as a screening method for detecting boar taint in a large numbers of boars.

8:13

Detection of errors in the NIR-analysis of meat components

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Near-infrared (NIR) reflectance spectrophotometers with multivariate computer programs have been available for several years, and have also found applications in the analysis of meat products. The indirect calibration techniques used in NIR-analysis, ideally require that the range of calibration samples must represent all future unknown samples of that type. The calibration and prediction samples should also be measured under the same experimental conditions. Regular controls and adjustments of the calibrations are necessary to keep them accurate and reliable over a period of time.
Analysis of meat components (fat, water and protein) by NIR-spectroscopy has been used in the process control by a Norwegian meat processing company since 1980. Recent controls of the performance of the overall analysis on raw comminuted meat samples, showed increased standard errors of prediction (SEP), which indicates lower accuracy of the analysis.
The relationships between the measurements from the NIR- and standard analysis were graphically and statistically examined. Systematic biases and proportional errors in the NIR-predictions were thus discovered. In addition, certain individual samples yielded abnormally large differences between chemically and NIR-determined results. Whether these abnormalities were due to errors in the chemical control data or in the NIR-determinations was studied by inspection of the sum of fat, water and protein for chemically and NIR-determined data, respectively. The results showed that a reduced accuracy of the water analysis appears to be caused by errors in the standard analysis of water.
The standard analysis underestimated the water content in a number of samples, which indicated incomplete drying of the samples. This effect was more pronounced in high fat samples. Special attention must therefore be paid to the execution of the standard methods in the calibration and subsequent prediction control of NIR-instruments.

8:14

A polarographic method for determining heavy metals in raw materials and in meat and poultry products

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A method for determining lead, cadmium, copper and zinc in the same sample of a meat or poultry product mineralized with dry ashing, has been suggested, based upon A.C. square-wave polarography, using hydrochloric acid, orthophosphoric acid, perchloric acid, and ammonium chloride-ammonia mixtures as supporting electrolytes.

The method has been tested by adding metals to various meats and meat and poultry products. The recovery of all added metals ranged within 87-105% with the relative standard deviation being 0.009 to 0.18.

Based upon the results of analyses of long-stored canned meats, no significant differences (P=0.95) were found in the amount of lead, copper and zinc measured by the suggested method and atomic absorption spectrophotometry.