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# Biogenic amines in meat meal
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# Introduction

Biogenic amines occur naturally and are also formed by bacterial conversion of free amino acids, especially in meat byproducts. There is increasing evidence and a general perception that biogenic amines are responsible for losses in animals and poultry consuming diets that contain meatmeal. However there is little data on the concentrations of these compounds in meatmeals or their possible interactions with other feed components. Limited attention is given to incorporating strategies into meatmeal processing that will reduce the formation of biogenic amines. Moreover, dietary biogenic amines may result in toxic effects in humans and a limit of 100 mg histamine/kg has been set for food destined for human consumption by the National Food Authority.

The aims of the project are to determine the scope of the problem of biogenic amines in meatmeals and to suggest possible strategies to minimize their occurrence. These objectives will be achieved by the following activities;

1. Comprehensive literature review of the occurrence and biological effects of biogenic amines.

2. Compilation of a data base of the concentrations of biogenic amines in Australian meatmeals using HPLC.

3. Pilot plant studies to evaluate the influence of various processing conditions on biogenic amine occurrence in meatmeals and determine opportunities in this area to optimise rendering to reduce biogenic amine production.

Preliminary results of the project have been compiled under 4 sections:

1. Computer Data Base Search and Review

2. Industry Survey

3. Survey of Selected Renders

4. Pilot Plant Processing

# 1. Computer Data Base Search and Review

## Literature Summary

Biogenic amines, of which the polyamine spermine was first crystallised by Antonie van Leeuwenhoek from human semen some 300 years ago, occur in all living organisms. The ubiquitous occurrence of these metabolites implies important biological functions. However such unsavoury names as putrescine and cadaverine for some of the amines has not helped the general profile of these compounds but recent suggestions of their possible role in carcinogenesis have caused considerable interest. Moreover, although necessary for growth, biogenic amines can also be toxic.

Biogenic amines occur at low concentrations in all cells and generally arise from the decarboxylation of amino acids. In aged, fermented or putrefied food and meat products much higher concentrations may occur due to the action of microorganisms. Any free amino acids in spoiled commodities can be broken down by means of deamination (production of ammonia) or decarboxylation by microbial decarboxylases (production of biogenic amines). This latter aspect is of importance to renders as an increase in biogenic amine content of meat products, especially meatmeals, indicates a decrease in protein quality through the loss of amino acids and an increase in the concentration of potentially toxic compounds, both undesirable in animal nutrition.

### Occurrence of Biogenic Amines

As would be expected from the ubiquitous occurrence of biogenic amines in cells, these compounds are found in all foods. The most important biogenic amines occurring in foods are the heterocylic amines histamine and tryptamine, aromatic amines such as tyramine and phenylethylamine, the diamines putrescine and cadaverine, and the polyamines spermine and spermidine.

Most studies of biogenic amine concentrations in food have concentrated on fish and cheeses because of the association of biogenic amine (especially histamine) poisoning with these foods. Concentrations of 0-5000 mg histamine/kg and 0-3000 mg tyramine/kg have been reported in fish and fish products. A limit of 100 mg histamine/kg has been set in Australia for food destined for human consumption by the National Food Authority. In a recent survey of fish and fish products in Melbourne, 15% of samples exceeded this level. Analysis of animal protein meals by the same group found the highest levels of histamine, putrescine and cadaverine occurred in fish meal.

Central to food and feedstuff surveys are reliable analytical techniques and several quantitative methods for the determination of biogenic amines in various commodities have been developed. At present, very sensitive HPLC methods are the techniques of choice for amine analysis.

### Biological Functions of Biogenic Amines

In addition to being precursors for the synthesis of hormones, nucleic acids, neurotransmitters and proteins, amines are important food aroma components and also

potential precursors for the formation of carcinogenic N-nitroso compounds. Cases of food intoxication have been mainly due to monoamines such as histamine, tyramine, phenylethylamine and tryptamine. Some amines, especially di- and polyamines, have also been proposed as food spoilage indicators.

Biologically active amines can be classified by function: (1) vasoactive (pressor amines) including (a) blood pressure increasing tyramine, tryptamine, phenylethylamine and isoamylamine and (b) blood pressure reducing histamine and serotonine and (2) psychoactive including norepinephrine, serotonine and dopamine. The diamines putrescine and cadaverine as well as the polyamines spermine and spermidine can potentiate the effect of histamine and through their roles in the regulation of nucleic acid and protein synthesis and membrane function are essential for cell proliferation. For this reason, di- and polyamines are extensively studied in connection with cancer.

Studies in which biogenic amines were fed to calves, pigs and chickens have given variable results. In some instances biogenic amines have been shown to enhance growth and in other experiments to be toxic. The variation may be due to a number of factors including:

1. Dietary concentrations fed

2. Metabolic capacity

3. Physiological state

4. Interactions between biogenic amines

5. Toxicity threshold.

There is very little information on the relative importance of any of these factors in determining the outcome of ingestion of biogenic amines. The situation is very complex as polyamine requirements for growth that cannot be met by endogenous biosynthesis must be satisfied by exogenous polyamines derived from food. The food polyamines are directed preferentially to tissues and organs that have been stimulated to grow by metabolic signals.

## Spoilage and Biogenic Amine Formation

Only very small concentrations of biogenic amines occur in fresh foods and it has been proposed that increases in the concentration of these compounds may indicate spoilage, especially for fish, dairy products, meat and wine. Different biogenic amines have been proposed as indicators of spoilage in different foods. Significant correlations have been found between putrescine, cadaverine and spermidine concentrations and total microbial count in retail minced beef. Nevertheless, the relationship is not straightforward as the microbial contamination is not constant and the bacteria present may not produce the indicator biogenic amine. In addition, storage conditions and processing will influence the quantity and profile of amines produced. The following factors will determine amine production:

1. Presence of decarboxylase-positive microorganisms

2. The presence of a suitable cofactor and/or inducer of decarboxylation

3. Availability of a suitable substrate (e.g. free amino acids)

4. Composition of raw material

5. Environmental conditions favouring microbial growth and decarboxylase synthesis (especially temperature, pH and water activity).

Obviously, control of these factors should reduce the concentrations of biogenic amines in feedstuffs. With meatmeals, this could be achieved by incorporating strategies into rendering that reduce the formation of biogenic amines.

# Occurrence and Quantification of Biogenic Amines in Animal and Plant Materials and their Effects on Poultry and Swine Production

Bibliography of reference titles and abstracts

## Bioamines in Animal and Plant Materials and their Effects on Poultry and Swine Production

**Ababouch, L., M. E. Afilal, H. Benabdeljelil, and F. F. Busta. 1991.** Quantitative changes in bacteria, amino acids and biogenic amines in sardine (Sardina pilchardus) stored at ambient temperature (25-28°C) and in ice. *International Journal of Food Science and Technology* 26 (3): 297-306.

> Freshly caught sardines contained high levels of bacteria located mainly on the skin and the gills. These bacteria invaded and grew rapidly in sardine muscle, reaching 5X108 and 6X108 respectively, colony forming units/g, after 24 h at ambient temperature and 8 days in ice. Histidine, arginine, lysine, tyrosine and methionine levels decreased during storage. The other amino acids, except proline and taurine, accumulated in the fish muscle, indicating extensive proteolysis. Histamine, cadaverine and putrescine accumulated to levels of 2350, 1050 and 300 p.p.m., respectively, after 24 h storage at ambient temperature. Histamine and cadaverine reached similar levels after 8 days' storage in ice, whereas putrescine formation was insignificant. Spermidine and spermine levels increased slightly under ambient conditions. Salting the fish at 8% delayed bacterial and chemical changes, but only in iced sardines. The high content of free histidine found in sardines, and the susceptibility of its muscle to histamine and cadaverine formation, could explain its increasing implication in incidents of histamine poisoning.

**Alam Khan. 1990.** Soybeans in foods - III anti-nutritional factors with emphasis on phytic acid - an overview. *Sarhad Journal of Agriculture* 6 (6): 557-561.

> The antinutritional factors in soyabeans are classified and their mechanisms are briefly discussed in this review. It is concluded that heat processing inactivates trypsin inhibitors, haemagglutinins, goitrogens and lysinoalanine, thereby increasing the digestibility of soyabeans.

**Allison, C., and G. T. Macfarlane. 1989.** Influence of pH, nutrient availability, and growth rate on amine production by Bacteroides fragilis and Clostridium perfringens. *Applied and Environmental Microbiology* 55 (11): 2894-2898.

> Dimethylamine, methylamine, propylamine, and pyrrolidine were the major amines formed by Bacteroides fragilis NCDO 2217 during the active phase of growth in batch culture. Production of these metabolites was strongly pH dependent and was optimal pH 6.0. Low pH also favoured the formation of pyrrolidine, cadaverine, and dimethylamine by Clostridium perfringens C523, but the reverse was the case with putrescine, butylamine, and propylamine, where production was maximal at pH 7.0. B. fragilis was grown in starch- or casein-limited continuous culture. Amine formation was influenced by carbohydrate availability and was greatest when the bacteria were grown at high growth rates (dilution rate, 0.20/h) under starch limitation, where they formed about 18% of the total fermentation products measured. Amine production was optimal and increased concomitantly with growth rate when C. perfringens was grown in glucose-limited continuous culture. Under conditions of high growth rate and glucose limitation, amines formed about 30% of the fermentation products measured. When glucose was increased from 5 to 15 g/litre, amine production was depressed, and under these nutritional conditions the growth rate had little effect on the process.

**Alving, K., R. Matran, J. S. Lacroix, and J. M. Lundberg. 1990.** Capsaicin and histamine antagonist-sensitive mechanisms in the immediate allergic reaction of pig airways. *Acta Physiologica Scandinavica* 138 (1): 49-60.

> The airway vascular and bronchial responses to Ascaris, histamine and capsaicin aerosol, were studied in pigs sensitized with A. suum. Histamine was found to be one of the main vasodilatory mediators released upon allergen challenge.

**Annan, W. D., and Manson W. 1981.** The production of lysinoalanine and related substances during processing of proteins. *Food Chemistry* 6 (3): 255-261.

> Processing foods may cause unintended changes in their constituent proteins. Studies on bovine milk proteins, including alphas0-, alphas1- and beta-casein, have assisted in understanding the formation of

lysinoalanine, which has toxic properties, and some phosphoproteins. Protein lysyl residues may enter the Maillard reaction with degradation products of carbohydrates, but losses of lysine may occur in the absence of free carbohydrate. Other alanines have also been detected. In specific caseins with 0.2 M NaOH, formation of lysinoalanine corresponded to the lysine loss but not to serine loss. Cyanate, formed from milk urea, may lead to homocitrulline formation.

**Anonymous. 1990.** Histamine: a neurotransmitter that influences food intake? *Nutrition Reviews* 48 (12): 439-440.

A brief review of recent results indicates that brain histamine levels in rats in various states of water balance were raised by blocking histamine catabolism with metoprine. Acute administration of this drug caused a significant reduction in food intake. This finding suggested a possible role of histamine in the regulation of food intake.

**Anonymous.1992.** Is putrescine an essential nutrient for avians. *Nutr- Rev. 50 (3): 81-83.*

Putrescine addition to a complete crystalline amino acid diet elicited a growth response in young chicks, suggesting that putrescine may be an essential nutrient in avians. However, high dietary putrescine levels can depress weight gain.

**Anonymous.**1976. Processed protein foods and lysinoalanine. *Nutrition Reviews* 34 (4): 120-122.

Lysinoalanine, a nephrotoxic amino acid formed upon alkaline treatment of protein, is apparently widely distributed in cooked, high-protein foods. However, only the free amino acid seems to be toxic, so that its presence in protein is mitigated by the probability that it is not released by the normal digestive process.

**Arnold, S. H., and Brown W. D. 1978.** Histamine (?) toxicity from fish products. *Advances in Food Research* 24: 113-154.

This comprehensive review first discusses the nature of the problem, symptomology, and cases of histamine toxicity from eating fish or fish products, then reviews mechanisms of the formation of histamine in fish (by 'autolytic' enzymes, detection of bacterial histidine decarboxylases, bacteria responsible for histamine formation, occurrence of histamine formers, free histidine as a histamine precursor, histidine decarboxylase, bacterial destruction of histamine, and 'saurine'); methods for detection and detn. of levels of histamine in fish, i.e. by guinea pig ileum contraction, other bioassays, fluorometric assay, GLC, colorimetric assay, enzymic isotopic assay, and TLC; relationship of spoilage to histamine formation; and unresolved problems, i.e. is scombroid toxicity due to histamine?, the need for improved analytical procedures, the question of anserine and carnosine, possible synergists or potentiators, and allowable levels of histamine in fish (the max. level most frequently discussed unofficially is about 10 mg/100 g in tuna fish).

**Arnold, S. H., Price R.J., and Brown W.D. 1980.** Histamine formation by bacteria isolated from skipjack tuna, Katsuwonus pelamis. *Bulletin of the Japanese Society of Scientific Fisheries [Nihon Suisan Gakkai shi]* 46 (8): 991-995.

A simplified method for measuring histamine formation in bacterial cultures is described. Histamine production by Proteus morganii, Proteus vulgaris and Hafnia alvei cultures isolated from spoiled skipjack tuna was measured under 12 environmental conditions. The highest histamine concn. were found at 19 degree C and 30 degree C depending on the bacterial spp. and the environmental parameter. No histamine was formed at 1 degree C, indicating that the rapid cooling of tuna flesh to freezing temp. may adequately suppress histamine formation. At 19 degree C and 30 degree C, the Proteus organisms at first formed high levels of histamine, much of which was subsequently destroyed; histamine concn. in these cultures eventually stabilized at 150-200 mg/100 ml. These observations indicate that the concn. of histamine in tuna products may depend on an equilibrium between histamine production and destruction.

**Askar A. 1973.** [Amines in fruits and vegetables and in their products (including wine). I.] Amine in Fruechten, Gemuese und deren Produkten. I. *Chemie Mikrobiologie Technologie Der Lebensmittel* 2:65-70.

**Azudin, M. N., and N. Saari. 1990.** Histamine content in fermented and cured fish products in Malaysia. *FAO-Fisheries- Report. 1990, No. 401, suppl. Pages 105-111 for 7th Session of the Indo-Pacific Fishery Commission Working Party on Fish Technology and Marketing.*

Histamine contents of fermented and cured fish products in Malaysia were analysed using fluorometric estimation. A wide range of histamine levels was detected. Cured fish had histamine 1.0 to 85 mg% of fish flesh. Fermented fish products ('cincaluk' and 'belacan') had high histamine content (>50 mg%). Formation of histamine during the processing of these fermented and cured fish products was also studied. Causes and economic implications of high histamine contents are discussed.

**Bailey, C. J., and Averill I. 1978.** The occurrence of 2-aminoethanol in food hydrolysates. *Irish Journal of Food Science and Technology* 2 (2): 123-128.

Ground barley samples were hydrolysed and the basic amino acids determined by an amino acid analyser after chromatography of the filtered hydrolysates. An unusual ninhydrin-positive substance which gave a small peak close to that of NH3 on the chromatogram was isolated by electrophoresis and identified as 2-aminoethanol. Further studies indicated that this compound is released from the lipid fraction of barley but that the amount present is related to the protein content. It is suggested that 2-aminoethanol is quantitatively derived from phosphoethanolamine. The 2- aminoethanol content of various food hydrolysates was determined; results were as follows (mumol/g): barley 2.1-3.0, wheat 1.6, maize 0.58, wheat pollard 1.2, soybean meal 3.7, groundnut meal 0.65, sunflower meal 1.5, fishmeal 3.1.

**Baldrati G, M. B. Fornari, Spotti E., and Incerti I. 1980.** [Effect of temperature on histamine formation in fish with high free histidine contents.]. *Industria Conserve* 55 (2): 114-122.

The effects of storage time and temp. on histamine formation were studied in fresh mackerel and after 1 day storage at 30 degree C, 1 and 3 day at 18 degree C, 3, 4 and 5 days at 4 degree C and 5, 14 and 30 days at -18 degree C. Results, shown graphically and in tables, revealed most extensive histamine production at 18 degree C (66.7 mg/100 g after 3 days), less at - 18 degree C (15.3 mg/100 g after 30 days) and 4 degree C (23.9 mg/100 g after 5 days) and negligible formation at 30 degree C (1.8 mg/100 g after 1 day). No relation was observed between total aerobe count and histamine contents of the fish.

**Bamba, T., K. Fuse, H. Obata, M. Sasaki, and S. Hosoda. 1993.** Effects of small peptides as intraluminal substrates on transport carriers for amino acids and peptides. *Journal of Clinical Biochemistry and Nutrition* 15 (1): 33-42.

The effects of small peptides on brush-border membrane enzyme activities and on transport carriers for amino acids and dipeptides were investigated by measurement of these enzyme activities and the transmural potential difference in the small intestine of guinea pigs fed on elemental diets containing small peptides (SP) or amino acids (AA) as the nitrogen source. Brush- border membrane aminopeptidase activites were significantly higher in the group fed on the diet containing SP. In addition, the transmural potential induced by L-leucine and glycyl-L- leucine (Gly- Leu) were significantly higher in the SP group. The maximum potential differences for L-leucine and Gly-Leu were significantly higher in the SP group, whereas the half saturation values did not differ between groups. The same effect of a SP diet was seen in rats, when the uptake of L-[U- 14C]leucylglycine (L-[14C]Leu-Gly) into intestinal brush-border membrane vesicles was measured. 70% of L-[14C]Leu-Gly was not hydrolyzed by aminopeptidase, but was found as a dipeptide in the brush-order membrane vesicles. These results indicate that small peptides as intraluminal substrates increase brush-border membrane aminopeptidase activities and the activity of carriers for amino acids and dipeptides.

**Bardocz, S., Brown, D. S., Grant, G. and Pusztai, A. 1990.** Luminal and basolateral polyamine uptake by rat small intestine stimulated to grow by phaseolus-vulgaris lectin phytohemagglutinin in-vivo. *Biochim. Biophy. Acta* 1034: 46-52.

Luminal and basolateral uptake of polyamines by the rat small intestine was studied in vivo. In the concentration range studied (0.1-5 mg per rat) 23-47% of the individual polyamines given intragastrically were found in the body after 1 h, with the small intestine retaining 4-12% of the dose. With spermidine or spermine, labelled polyamines accounted for 85-96% of the counts in the small intestine and between 72-82% were in the form given. However, with putrescine only 29-39% of the label found in the tissue remained in polyamine form and even less, 11-15%, as putrescine. Luminal uptake of polyamines was linear, non-saturable and was not stimulated when small intestinal growth was stimulated by phytohaemagglutanin (PHA). On the basolateral side of the gut, polyamine uptake was stimulated by PHA in a time-dependent way in advance of detectable growth. Overall polyamine recoveries were high (89-99%) with intraperitoneally administered spermidine and spermine. Moreover, a large proportion of the counts in the tissue (63-89%) were still in the original form. Even with putrescine, total recoveries of polyamines (72-88%) and putrescine (24-33%) were elevated in comparison with those from the lumen. Treatment of rats with .alpha.-difluoromethylornithine (DFMO), an inhibitor of ornithine decarboxylase, reduced tissue polyamine content, although it had slight effects only on basolateral polyamine transport. The PHA-stimulated increase of polyamine uptake was not abolished in the presence of DFMO.

**Bardocz S, Grant G, D. S. Brown, Ralph A., and Pusztai A. 1993.** Polyamines in food - implications for growth and health. *Journal of Nutritional Biochemistry* 4 (2): 66-71.

Different types of food (fruits, vegetables, meat and dairy products) were analysed by HPLC to determine their polyamine (putrescine, spermidine and spermine) contents. Quantitative data on the polyamine composition of foods is presented. All foods contained some polyamines, although the concn. in different individual food components were variable. As was established earlier using -1-4C-labelled putrescine, spermidine and spermine, polyamines are readily taken up by the gut and enter the systemic circulation. Food appears to constitute a major source of polyamines for humans and animals. Distribution of polyamines in the body, as determined by measuring the accumulation of -1-4C-spermidine in different tissues of the rat, was correlated with the metabolic activity and growth of particular organs. It was concluded that food polyamines are not only necessary for normal body metabolism, but are also used and directed preferentially to tissues and organs that have been stimulated to grow by metabolic signals. [From En summ.].

**Bateman, R. C. ,. Jr., D. B. Eldrige, S. Wade, J. McCoy Messer, E. L. E. Jester, and D. E. Mowdy. 1994.** Copper chelation assay for histamine in tuna. *Journal of Food Science* 59 (3): 517- 518, 543.

A reported copper chelation method for histamine estimation was modified for use in decomposing yellowfin tuna steaks. The assay consisted of histamine extraction with hot methanol, purification by rapid cation exchange chromatography, and addition of copper and a dye to the purified sample to form an easily visualized red complex with the histamine. This method detected histamine in the low mg% range in yellowfin tuna steaks with an accuracy comparable to the standard fluorometric histamine assay. It is an alternative to the standard assay when a visual test is desirable or a fluorometer is not available.

**Bauer, F., R. Tschabrun, and K. Sick. 1989.** Histamine in Austrian long-ripened dry sausages. *Wiener Tierarztliche Monatsschrift* 76 (6): 180-184.

Between 1985 and 1987, 129 samples of dry sausages matured for up 12 weeks (such as salami) of Austrian origin were tested for histamine by fluorometry according to paragraph 35 of the Austrian Federal Health Regulations (1982). Amounts of between 1 and 654 mg/kg DM were detected. In more than half the samples, histamine concentrations were below 100 mg/kg. Different manufacturing methods did not influence histamine formation (except in thin products), but there were highly significant differences in dry sausage products from different manufacturers.

**Bedford, M. R., T. K. Smith, and J. D. Summers. 1987.** Effect of dietary lysine on polyamine synthesis in the chick. *Journal of Nutrition* 117 (11): 1852-1858.

The effect of dietary lysine on hepatic and renal polyamine synthesis was studied in the chick. High-arginine-requiring (HA) and low-arginine- requiring (LA) strains were used, with differences in arginine requirements being due, in part, to difference.

**Bjeldanes, L. F., Schutz D.E., and Morris M.M. 1978.** On the aetiology of scombroid poisoning: cadaverine potentiation of histamine toxicity in the guinea-pig. *Food and Cosmetics Toxicology* 16 (2): 157-159.

Sporadic outbreaks of human poisoning have occurred following ingestion of scombroid fish, such as, bonito, mackerel and tuna. Histamine is considered to be involved in scombroid poisoning, but it does not appear to be the only causative agent. The peroral toxicity of histamine in the guinea-pig is shown to be potentiated by simultaneous administration of cadaverine. The relative levels of cadaverine and histamine present in toxic fish are of the same order of magnitude as those that show potentiation of histamine toxicity in the guinea-pig. On the basis of currently available evidence, it seems likely that cadaverine, along with histamine, is of importance in the aetiology of scombroid poisoning.

**Blonz, E. R., and Olcott HS. 1978.** Effect of histamine, putrescine and of canned spoiled tuna on growth in young Japanese quail. *Journal of Food Science* 43 (5): 1390-1391.

Newly hatched quail were fed for 9 days with chick starter meal, or diets based upon 60% freeze-dried spoiled or nonspoiled tuna. The diets were fed alone, or with added histamine or putrescine. Histamine in the chick starter meal depressed wt. gain, while the putrescine had no apparent effect at the level used (2.2 g/kg diet). The diets based upon 60% freeze-dried spoiled tuna significantly depressed wt. gain when compared to the nonspoiled tuna diets. No significant effect was observed when histamine was added to nonspoiled tuna diets at levels equalling or exceeding that in the spoiled fish. Quail might be useful bioassay animals for the isolation and identification of toxic factors in canned spoiled tuna fish.

**Blonz, E. R., and H. S. Olcott. 1978.** Effects of orally ingested histamine and/or commercially canned spoiled skipjack tuna on pigs, cats, dogs and rabbits. *Comp. Biochem. Physiol. C. 61 (1): 161-163.*

**Bordallo Costas, F., and A. Lamela Gonzalez. 1989.** Estimation of histamine in fish by combined thin-layer chromatography and high-performance liquid chromatography. *Alimentaria* 26 (207): 39-40.

A method combining thin-layer chromatography and high- performance liquid chromatography to estimate histamine in fish products is described.

**Boulton, A. A., Cookson, B. and Paulton. R. 1970.** Hypertensive crisis in a patient on mono amine oxidase inhibitor anti depressants following a meal of beef liver. *Can. Med. Assco. J.* 102: 1394-1395.

**Brugh, M., and R. L. Wilson. 1986.** Effect of dietary histamine on broiler chickens infected with avian reovirus S1133. *Avian Diseases* 30 (1): 199-203.

One-day-old broiler chicks were infected with one of two vaccine strains of avian reovirus S1133 and fed diets containing 0.2% histamine dihydrochloride for 21 or 35 days. Control groups received either or neither of these treatments. The most notable virus-histamine interaction was increased early mortality of chickens infected with the more virulent (pullet) vaccine virus. Histamine in the diet did not affect seroconversion rates or the incidence of stunting in virus-infected chickens. Other evidence of virus-histamine interaction was proventricular enlargement and decreased weight gains in chickens infected with the less virulent (chick) vaccine virus, but these signs were observed inconsistently. The possible clinical significance of these observations is discussed.

**Bryan, F. L. 1988.** Risks associated with vehicles of foodborne pathogens and toxins. *Journal of Food Protection* 51 (6): 498-508.

Annual US foodborne disease surveillance reports for 1977-1984 are reviewed and results presented in tabular form. The main foods associated with scombroid (histamine) poisoning, ciguatera poisoning, Clostridium perfringens enteritis, salmonellosis, staphylococcal food poisoning, shigellosis, botulism and Bacillus cereus gastroenteritis are listed. Literature relating to outbreaks of food poisoning caused by the different categories of foods is also discussed in detail, with consideration of various mechanisms of disease. Seafoods (24.8% of outbreaks), meats (23.2%), poultry (9.8%) and salads (8.8%) were the most frequent sources of illness. The most frequently implicated individual food items were roast beef, ham, turkey, chicken and raw clams. Recommendations are made concerning follow-up studies on food poisoning outbreaks.

**Buckley, N. M., S. Diamant, I. D. Fraiser, and K. Owusu. 1988.** Histamine or adenosine blockade alters intestinal blood flow autoregulation in swine. *American Journal of Physiology* 254 (2): G156-G161.

The possible role of histamine or adenosine in intestinal blood flow autoregulation in 10 month-old swine was examined by obtaining pressure- flow relationships before and during intestinal histamine H1- or adenosine-receptor blockade in two groups of fasting animals under anaesthesia with pentobarbital sodium (30 mg/kg). Changes in abdominal and thoracic aortic pressures and in superior mesenteric and left renal arterial flows were recorded during controlled aortic compression above the coeliac artery. After control intestinal and renal pressure- flow relationships were obtained, a test dose of agonist (0.1 µg histamine or 0.2 µg adenosine/kg body wt) was given into the superior mesenteric artery. Then an intra- arterial infusion of blocking agent was started (0.1 mg/kg/min chlorpheniramine or 10 µmol/min theophylline). Degree of blockade was assessed with doses of agonist given before and after a second set of intestinal and renal pressure-flow relationships was obtained. Complete blockade of intestinal and renal pressure-flow relationships was obtained. Complete blockade of intestinal vascular histamine H1-receptors with chlorpheniramine abolished, and incomplete blockade of adenosine-receptors with theophylline attenuated, intestinal blood flow autoregulation. Renal blood flow autoregulation remained at its control level. These results indicate that both histamine and adenosine are among the physiological vasodilators contributing to intestinal blood flow autoregulation when arterial pressure is decreased in young swine.

**Burrin, D. G., R. J. Shulman, C. Langston, and M. C. Storm. 1994.** Supplemental alanylglutamine, organ growth, and nitrogen metabolism in neonatal pigs fed by total parenteral nutrition. *Journal of Parenteral and Enteral Nutrition* 18 (4): 313-319.

Organ growth, intestinal enzyme activity, and plasma nitrogen metabolites were compared in 4-day old pigs randomly selected to receive total parenteral nutrition (TPN) supplemented with glutamine 0, 2.0, or 4.5 g/100 ml for a total amino intake of 11.

**Butikofer, U., D. Fuchs, D. Hurni, and J. O. Bosset. 1990.** Determination of biogenic amines in cheese. Comparison of an improved HPLC method with an IC method, and application to different cheeses. *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene* 81 (1): 120-133.

A modified version of Etter's HPLC method [ibid. 106] gave very good agreement (r>0.99) with a routine ion-chromatographic method [Schweizerische Milchwirtschaftliche Forschung 14 (3) 3] for putrescine, cadaverine, histamine and tyramine in Gruyere, Emmental, Tilsit, Gorgonzola, Appenzell and Camembert cheese. Agreement for beta-phenylethylamine was slightly poorer (r = 0.92). When 12 cheese varieties were screened by the HPLC method, highest amine contents were found in Gorgonzola (putrescine 164, cadaverine 1200 mg/kg) and Gorgonzola/ Mascarpone (tyramine 1560 mg/kg).

**Buts, J. P., N. Keyser de, J. Kolanowski, E. Sokal, F. Hoof van, N. De Keyser, and F. Van Hoof. 1993.** Maturation of villus and crypt cell functions in rat small intestine. Role of dietary polyamines. *Digestive Diseases and Sciences* 38 (6): 1091-1098.

To evaluate the role of dietary polyamines in maturation of rat small intestine, spermine was given orally twice daily to sucking Wistar rats from day 10 to 14 postpartum at different doses: 0, 0.2, 0.5, 1, 2.5 and 5 µmol. Compared with saline treated controls, spermine (5 µmol) produced increases in mucosal mass parameters (12 to 57%, P<0.05), induced prematurely an adult pattern of microvillous enzymes and enhanced, respectively, by 19- and 3.5-fold (P<0.01 vs. controls) the concentration of the secretory component of p-immunoglobulins in villous and crypt cells. The response of microvillous enzymes (lactase, sucrase, maltase and aminopeptidase) to spermine was dose-dependent and - specific since oral administration of arginine (5 µmol) or ornithine (5 µmol) was without effect. Intestinal changes were significant (P<0.05) for doses of spermine >1 undetectable at the physiological amounts of polyamines consumed by young rats from rat milk during the sucking period (<0.3 µmol/day). Consistent with a direct effect of spermine on the intestinal cell, cytosolic activity of ornithine decarboxylase was depressed 27-fold (P<0.005 vs. controls) in the jejunum, while inhibition of ornithine decarboxylase by alpha-difluoromethylornithine markedly decreased but did not suppress cell response to spermine. Plasma corticosteroanemia, which was virtually absent by day 14 in controls, ranged from 1.4 to 4.6 µg/100 ml in 60% (n=9) of spermine-treated rats. Findings indicate that dietary polyamines exert direct and indirect trophic effects on the rat immature intestine and can trigger at a critical level of intake the adult expression of villus and crypt cell functions.

**Cabrera Saadoun, M. C., and B. Sauveur. 1987.** Hyperphosphataemia and bone resorption in histamine injected laying hens. Comp. Biochem. Physiol. A 87 (1): 183-187.

The effects of histamine on bone resorption during egg shell formation, were studied, including the effect of histamine on the clearance of 45calcium and 32phosphorus from plasma, and on the release from the femur or uptake by the egg shell of 45Ca and 32P injected at the time of the first experiment or 10 h before the experiment (experiment 2). Histamine decreased the disappearance rates of 45Ca and 32P, without any modification of femur and egg shell values. The increase in the amount of plasma 32P and the decrease in the 32P specific activity in bone elicited by histamine, argues in favour of the skeletal origin of hyperphosphataemia induced by histamine during egg shell formation.

**Calonge, M. L., A. Ilundain, and J. Bolufer. 1990.** Glycylsarcosine transport by epithelial cells isolated from chicken proximal cecum and rectum. Am. J. Physiol. 258 (5) : G6660-G6664.

**Campbell, R. M., and C. G. Scanes. 1988.** Pharmacological investigations on the lipolytic and antilipolytic effects of growth hormone (GH) in chicken adipose tissue in vitro: evidence for involvement of calcium and polyamines.

*Proc. Soc. Exp. Biol. Med.* 188 (2): 177- 184.

The involvement of RNA/protein synthesis, calcium, calmodulin, protein kinase C, and polyamines in the lipolytic and antilipolytic (inhibition of glucagon-stimulated lipolysis) responses to GH have been investigated employing chicken adipose tissue in.

**Carlucci, F. V., and Karmas E. 1988.** Liquid chromatographic determination of some amines and their amino acid precursors in protein foods. *J. Assoc. Offic. Anal. Chemists* 71 (3): 564-568.

A quantitative assay was developed for putrescine, cadaverine, histamine, and their precursor amino acids, lysine, arginine and histidine, as a measure of decomposition. The free amines and amino acids are extracted from tissue with methanol. Aliquots of the extracts are passed through ion-exchange columns which are pH-adjusted to retain the cited compounds. After the columns are washed, the amino acids and amines are eluted, dansylated, and chromatographed on a suitably prepared liquid chromatograph. Chromatographic responses for the amino acids and amines are compared with their respective standards, to determine their concn. Validation of the methodology includes standard addition and calibration experiments. The method may be used to quantitate other protein-related compounds, and on protein sources other than tissue protein.

**Carnegie, P. R., Collins MG, and Ilic MZ. 1984.** Use of histidine dipeptides to estimate the proportion of pig meat in processed meats. *Meat Science* 10 (2): 145-154.

Studies were conducted to evaluate concn. of histidine dipeptides (anserine, carnosine, balenine) as an index of the proportion of pork in processed meat products. A HPLC procedure for detn. of histidine peptides is described, based on extraction with 0.9% saline followed by 10% sulphosalicylic acid, filtration of the extract, and separation on a Whatman Partisil- 10 SCX column, elution with 0.2M lithium formate (pH 2.9). Eluates are reacted with o-phthaldialdehyde; detection is with a Waters fluorescence detector. An amino acid analyser with a sulphonated polystyrene column was also evaluated. The HPLC method was much faster; it was also more accurate, and had lower reagent costs. The HPLC method gave clear separation of the 3 peptides from amino acids and from each other. Data are given for concn. of total histidine peptides, anserine, carnosine and balenine, and for balenine/anserine and carnosine/anserine ratios in pork, chicken, beef, lamb and mutton; balenine concn. and balenine/anserine ratios were much higher in pork than in the other meats. Use of these characteristics for detn. of the proportion of pork in meat products is discussed. The results of a study on commercial products showed the following proportions to have an unsatisfactory pork content: 'pork' sausages 8 of 10; 'ham and chicken' luncheon meat 1 of 10; miscellaneous products labelled 'pork' or 'ham', 7 of 16. Additionally, many samples had low lean meat contents.

**Carnegie, P. R., Ilic MZ, Etheridge MO, and Collins MG. 1983.** Improved high-performance liquid chromatographic method for analysis of histidine dipeptides anserine, carnosine and balenine present in fresh meat. *Journal of Chromatography* 261 (1): 153-157.

In order to monitor rapidly the species of origin of meat used in meat products, a simpler and more rapid isocratic system for separation of the histidine dipeptides (HDP) present in muscle of meat animals was developed. HDP were extracted from lean muscle samples, and separated on a Partisil-10 SCX column with 0.2M lithium formate, pH 2.9 at 40 degree C under isocratic conditions with post-column derivatization with OPA reagent. Contents of total HDP, anserine (ans), carnosine (car) and balenine (bal) and ans:car:bal ratio are shown for meat from various spp. (pig, beef, buffalo, goat, lamb, sheep, horse, donkey, kangaroo and rabbit). Ratios of HDP were found to be characteristic of skeletal muscle of animals of the same or related sp., e.g. meat from horse, kangaroo or sheep could be distinguished from beef by the amount of ans and the ans:car ratio. However this method could not distinguish between meat from

buffalo and cattle, from horse and donkey, nor from kangaroo and rabbit. Application of the method to meat products has been carried out and results will be reported elsewhere.

**Castell, C. H., Neal W., and Smith B. 1970.** Formation of dimethylamine in stored frozen sea fish. *J. Fisheries Res. Board Can.* 27 (10): 1685-1690.

In cod fillets undergoing deterioration during frozen storage, the dimethylamine content increases (and not the trimethylamine content as previously reported). There was no evidence to show an accumulation of dimethylamine in the muscle of frozen scallops, lobster, or shrimp that were purposely held at relatively high storage temp. It is suggested that for fish of the family Gadidae, dimethylamine might be used as a measure of frozen-storage deterioration in much the same way as trimethylamine has been used as a measure of microbial spoilage in the unfrozen fish.

**Celano, G. V., C. Cafarchia, F. Buja, and G. Tiecco. 1992.** Determination of biogenic amines in cheese. *Industrie Alimentari* 31 (307): 764-766, 768.

79 samples of Italian cheese of various types were analysed for biogenic amines (phenylethylamine, tryptamine, tyramine, putrescine, cadaverine and histamine) by TLC. Predominant biogenic amines differed between cheese types and each cheese type tended to have a characteristic biogenic amine spectrum. Histamine predominated in Grana Padano and Parmesan cheese; tryptamine predominated in Provolone cheese and tyramine predominated in Pecorino cheese.

**Cerutti, G., Finoli, C., Peluzzi, S. and Vecchio, A.** 1985. Non-volatile amines in beer: origin and occurrence. *Monatsschrift Fuer Brauwissenschaft* 38: 296-299.

Studies were conducted on concn. of biogenic amines, i.e. (i) hordenine, (ii) cadaverine, (iii) putrescine, (iv) beta-phenylethylamine, (v) tryptamine, (vi) histamine and (vii) tyramine, in 40 beers from 11 Italian breweries, and 2 samples of hop extract. Tables of results are given. None of the beers contained (vi); all contained (i), at 0.3-4.7 p.p.m.; 4 (all from 1 brewery) contained (ii), at 0.1-0.5 p.p.m.; all contained (iii), at 1.8-7.5 p.p.m.; 11 contained (iv), at 0.2-0.7 p.p.m.; 5 beers (from 4 breweries) contained (v), at concn. of 1.5-7.5 p.p.m.; all beers contained (vii), at 0.3- 70.8 p.p.m. No biogenic amines were detected in any of the hop extracts. The results are discussed in detail; possible sources of some of the amines are discussed.

**Chand, N., and P. Eyre. 1978.** Effects of histamine on chicken airways : possible pulmonary allergy as disease etiology. Vet. Sci. Commun. 2 (1): 77-82.

**Chaytor, J. P., Crathorne B., and Saxby M.J. 1975.** The identification and significance of 2-phenylethylamine in foods. *J. Sci. Food Agric.* 26 (5): 593-598.

Techniques are described for the extraction and analysis of amines from foods, employing gas chromatography and MS. The presence of 2- phenylethylamine was demonstrated in samples of chocolate, cheese and certain red wines. Subsequent clinical trials have shown that this amine precipitates migraine attack in dietary migraine sufferers [see FSTA (1974) 6 11C369].

**Chiavari, G., G. C. Galletti, and P. Vitali. 1989.** HPLC determination of catabolic amines in silage using their dansyl derivatives and an electrochemical detector. *Chromatographia* 27 (5/6): 216-220.

The use of the electrochemical detector (EC) in the high performance liquid chromatography (HPLC) of dansyl derivatives of biogenic amines is reported. Isocratic and gradient elution patterns of synthetic mixtures of putrescine, cadaverine, 1,6- diaminohexane, tryptamine, histamine, tyramine, spermine and spermidine on a reversed phase column are shown. Hydrodynamic voltammograms of standard compounds are given. The detection limits of the EC are compared with those of ultraviolet and spectrofluorimetric detectors. A chromatographic profile of amines from the proteolytic catabolism of maize silage by Clostridia is shown.

**Chin, K. W., Garriga M.M., and D. D. Metcalfe. 1989.** The histamine content of oriental foods. *Food and Chemical Toxicology* 27 (5): 283-287.

Several of the symptoms of scombroid poisoning (i.e. histamine toxicity) resemble those observed in people suffering from Chinese restaurant syndrome. Therefore, the histamine content of representative Chinese cuisine, which included 31 common dishes, 12 condiments and 12 basic ingredients from several sources, was measured using a sensitive and specific radioenzymic assay. A further enzymic procedure involving diamine oxidase was used to verify that the substance measured was histamine. A total of 184 assays were performed on 57 samples in the study. High levels of histamine were found in the cheeses, which were used as positive controls (863.6 mug histamine/g blue cheese and 107.4 mug histamine/g Parmesan cheese), and in some common condiments, including tamari (2392.2 mug histamine/g sample) and one brand of soy sauce (220.4 mug histamine/g sample). The histamine content of 4 condiments and 3 common dishes was greater than 10 mug histamine/g sample, while 4 condiments and 16 common dishes contained less than 1 mug histamine/g sample. Calculations involving representative amounts of food that can be consumed at a typical oriental meal suggest that, in some cases, histamine intake may approach toxic levels. The results are discussed with regard to the possible role of histamine in reactions associated with restaurant meals.

**Chin, K. D. H., and P. E. Koehler. 1986.** Effect of salt concentration and incubation temperature on formation of histamine, phenethylamine, tryptamine and tyramine during miso fermentation. *Journal of Food Protection* 49 (6): 423-427.

Salt concn. and incubation temp., were examined for their effect on the formation of histamine, phenethylamine, tryptamine and tyramine during miso (soybean paste) fermentation. Misos containing 5 and 10% NaCl were prepared and incubated at 25 degree and 35 degree C. The effect of each factor was determined from the chemical and microbiological changes in the misos during fermentation. Salt level was a significant factor in the formation of amines. Higher amine levels were found in low-salt (5% NaCl) formulations than in high-salt (10% NaCl) misos. Incubation temp. within the range 25-35 degree C during fermentation had little effect on amine formation in misos.

**Choudhury, N., W. Hansen, D. Engesser, W. P. Hammes, and W. H. Holzapfel. 1990.** Formation of histamine and tyramine by lactic acid bacteria in decarboxylase assay medium. *Letters in Applied Microbiology* 11 (6): 278-281.

A modified decarboxylase assay medium (DCA medium) was used for studying the production of biogenic amines by Leuconostoc oenos DSM 20252 and 2 strains of Lactobacillus buchneri (Lb14 and St2A). The DCA medium contained histidine, lysine, ornithine and tyrosine as precursors of the respective biogenic amines. Under the experimental conditions both strains of L. buchneri produced greater than 90% of the max. amount of histamine within 24 h. Only tyramine was produced by L. oenos DSM 20252, accounting for 88% of the max. theoretical amount within 24 h.

**Cirilli, G., C. S. A. Cirilli, and B. Spottl. 1991.** Estimation of histamine in fish products by high performance liquid chromatography. *Industrie Alimentari* 30 (292): 371-374.

A rapid method of estimating histamine in fish products by high performance liquid chromatography is described. An improved mobile phase using acetonitrile allows preferential isolation and optimal resolution of the amine, eliminating interference from the extraction matrix. Sensitivity is 100 pg and 10 ng/20 µl using spectrofluorimetric and UV/vis detectors, respectively. Execution time is about 20 min. Repeatability and accuracy is good.

**Clifford, M. N., R. Walker, P. Ijomah, J. Wright, C. K. Murray, and R. Hardy. 1991.** Is there a role for amines other than histamines in the aetiology of scombrotoxicosis? *Food Additives and Contaminants* 8 (5): 641-651.

Mackerel fillets associated with an outbreak of scombrotoxicosis were analysed for their contents of cadaverine, histamine, putrescine, spermidine, spermine and tyramine and fed to informed, healthy volunteers of both sexes under medical supervision. Of the 86 fillets examined, 30 rapidly induced nausea/vomiting and/or diarrhoea when 50 g were consumed. The remaining fillets failed to provoke such symptoms, even though 17 of them were tested by volunteers proven to be susceptible to scombro-intoxication. Statistical analysis failed to detect any differences in amines content between fillets shown to be scombrotoxic and those failing to induce nausea/vomiting and/or diarrhoea, and failed also to establish any significant relationships between the amines doses and volunteer responses, even after manipulations to simulate additive or synergistic interactions. Accordingly, it is concluded that the contents of such amines in mackerel have little or no role in the aetiology of scombrotoxicosis.

**Coas, V., and L. Lepri. 1982.** Separation of histamine reverse phase by HPTLC and soap-TLC and its determination in tuna fish. ???? 114-119.

Screening for histamine in tuna fish depends on separation of histamine from other amino acids, peptides and biogenic amines. Reversed-phase HPTLC on RP-2 and RP-18 plates, alone or impregnated with an anionic surfactant, e.g. dodecylbenzene- sulphonic acid, and chromatography on layered ammonium tungstophosphate were studied as separation methods for 21 nitrogenous compounds. Rf values of these compounds on the several media are tabulated, and optimal conditions listed for each. 22 tuna fish and mackerel extracts were similarly analysed and the presence of histidine, glycine and lysine and absence of cadaverine and putrescine observed; histamine could be unequivocally determined at 5-12 mg/100 g fish, levels well below those likely to be toxic (100 mg/100 g fish).

**Colnago, G. L., and L. S. Jensen. 1992.** Research note: putrescine effects on performance of male broiler chicks fed low-protein diets supplemented with essential amino acids. *Poult. Sci. 71 (1): 211-214.*

The purpose of the present study was to determine whether a polyamine deficiency could account for the reduced performance of broiler chicks fed a low-protein diet supplemented with several synthetic amino acids in comparison with those fed a more conventional CP concentration. Male broiler chicks were fed equicaloric diets calculated to contain 23, 20, or 17% CP. Each diet was supplemented with 0, .05, or .10% putrescine in a 3 X 3 factorial arrangement of treatments. Body weight gain of chicks fed the 17% CP diet was significantly ($P < .05$) less than that of those fed the higher protein diets and feed conversion ratio significantly increased as the CP concentration decreased. Adding putrescine failed to significantly affect any of the parameters within each CP concentration. A deficiency of polyamines does not appear to explain the lower performance of broiler chicks fed low CP diets with added synthetic amino acids. Universidade Federal Fluminense, Niteroi, RJ, Brasil.

**Cowan, J.C., Rackis, J.J. and Wolf, W.J. 1973.** Soybean protein flavor components: A review. *J. Amer. Oil Chemists' Soc.* 50: 426A, 428A, 430A, 432A, 434A-435A, 444A.

This review on flavour components of soybean products covers studies on sensory evaluation of commercial flours, concentrates and isolates, extraction of flavour components from soybean flakes with hexane-alcohol azeotropic mixtures, application of proteolytic enzymes to improve flavour, and effects of inactivation of lipoxygenase on the flavour of soy beverages. Evidence indicates that enzymic reactions affect the flavour of the final products. Presumably lipoxygenase is a primary culprit and linoleic acid a primary precursor when soybeans are partially processed before destroying enzymatic activity.

**Cowey, C. B., and C. Y. Cho. 1992.** Failure of dietary putrescine to enhance the growth of rainbow trout (Oncorhynchus mykiss). *Canadian Journal of Fisheries and Aquatic Sciences* 49 (12): 2469-2473.

Reduction of herring meal to 100 g/kg in rainbow trout (Oncorhynchus mykiss) diets and its replacement by soyabean and maize gluten meals reduced feed intake and growth. Putrescine 1 to 4 g/kg added to trout diets did not increase feed intake or growth over 12 weeks . Putrescine concentrations in the trout tissues were not increased by this treatment, nor were the activities of the putrescine and polyamine biosynthetic enzymes ornithine decarboxylase and adenosylmethionine decarboxylase significantly changed. Putrescine 13.3 g/kg a concentration similar to that used in calf diets, reduced weight gain, feed intake and gain/feed ratio. Lack of effect of putrescine at the lower concentrations (effective in chick diets) was ascribed to the enzyme diamine oxidase, shown to be active in the pyloric caeca and the anterior intestine of the trout. Passage of feed through the gastrointestinal tract of trout is about 10-fold longer than in chicks. This may explain the failure of dietary putrescine to increase putrescine concentrations in trout.

**Cozzani, R., A. Ubaldi, D. Barchi, A. Lullo di, and A. Di Lullo. 1990.** Histamine, putrescine and cadaverine in preserved fish products. *Industrie Alimentari* 29 (288): 1113-1116.

The concentrations of histamine, putrescine and cadaverine were estimated in 177 samples of Scombridae (mackerel and tuna fishes), Clupeidae (sardines and herrings) and Engraulidae (anchovies). Less than 10% of the samples had a level of histamine higher than the allowed limit. The largest proportion of non-regular samples was found in anchovies (37%) compared with mackerel (2.9%) and tuna fish (0%). Organoleptic qualities were normal in all cases. No correlation was found between histamine, putrescine and cadaverine concentrations.

**Dallyn, H. and Everton, J.R. 1973.** The influence of packaging materials on microbial growth. *Food Technology in Australia* 25: 436-438, 440-441, 443, 445.

In this review the author gives data on the permeability to O2, CO2, and H2O vapour of various packaging materials, effects of the packaging material (metal, plastics) on microbial growth, microbiology of packaged meat, plastics packages for cheese and low-moisture products, product protection of packaging material against microbial attack, and loss of preservatives as the result of flexible packaging. It is concluded that microbial growth is a major factor in the selection of the most suitable packaging material for a particular product.

**Davies, R. L. 1993.** D-Lysine, alloisoleucine and lysinoalanine in supplementary proteins with different lysine availabilities. *Journal of the Science of Food and Agriculture* 61 (2): 151-154.

D-Lysine, alloisoleucine and lysinoalanine were estimated in 16 commercial protein supplements for which lysine availability had been measured by slope-ratio assay with pigs and by chemical dinitrophenylation.

About 2.5% of lysine was racemised by protein hydrolysis in 6 M HCl. Only 3 of 10 samples with poor lysine availability by slope-ratio assay contained significantly more D- lysine than control proteins ($P<0.01$). D-Lysine was not significantly correlated with lysine availability by either method; nor did it improve the poor correlation between the slope-ratio assay and dinitrophenylation. The highest level of alloisoleucine was less than 1.4% that of isoleucine. In all proteins except dried skim milk lysinoalanine occurred at 0.3% or less of the corresponding lysine level. Neither alloisoleucine nor lysinoalanine was related to lysine availability.

**Debruyckere, G., and C. Peteghem van. 1992.** HPLC analysis of organic bases. *Food analysis by HPLC [edited by Nollet, L. M. L.] 52: 643-671.*

The definition of organic bases is outlined and the HPLC analysis of amines (biogenic amines and alkylamines), purine and pyrimidine bases, methylxanthines and various alkaloids in foods and beverages is discussed.

**Degheidi, M. A., B. A. Effat, and A. R. Shalaby. 1992.** Development of some biogenic amines during Ras cheese ripening with special reference to different starters. *Pages 205-217 in Proceedings 5th Egyptian Conference for Dairy Science and Technology.*

Ras cheese was made from heat-treated (71°C/30 s) cow milk using 1% of the following starters: (i) 1:1 Streptococcus salivarius var. thermophilus (ST):Lactobacillus delbrueckii var. bulgaricus (LB); (ii) 1:1 L. casei (LC):LB; and (iii) 1:1:1 LC:ST:LB. During ripening at 12°C for 5 months, all cheeses showed a decrease in moisture, and increases in acidity, salt-in-DM, total N (TN), soluble N:TN, non-protein N:TN, amino N:TN and ripening index. Histamine, putrescine and cadaverine were detected in all cheeses after ripening for 1 month, and reached max. levels after ≥5, 3 and 2-3 months resp. Tryptamine was not detected in any cheeses, whereas tyramine and phenylalanine were both detected after 2 and 3 months in cheeses made with (ii) and (iii) resp. Total viable and lactic acid bacterial counts decreased throughout ripening, proteolytic counts increased gradually up to the 4th month then rapidly declined, and coliforms were not detected except in cheese made with (ii) in which they decreased from 750/g initially and were not detected beyond the 2nd month. Max. organoleptic scores recorded for cheeses made with (i), (ii) and (iii) resp. were 87, 89 and 94 after 5, 5 and 3 months. Extending Ras cheese ripening to >3 months is not recommended.

**Dierick, N. A., I. J. Vervaeke, J. A. Decuypere, and H. K. Henderickx. 1986.** Influence of the gut flora and of some growth- promoting feed additives on nitrogen metabolism in pigs. 1. Studies in vitro. *Livestock Production Science* 14 (2): 161-176.

Nine growing pigs (10-40 kg) were fitted with a cannula either in the duodenum or ileum or caecum and fed on a dry-milk powder diet. These pigs were used as donors of fresh intestinal contents for short (4 h) incubation experiments. From the incubations of duodenal contents, it appeared that the bacterial activity was not important. In consequence, the antibiotic effects of Virginiamycin and Spiramycin were also negligible. During incubations of ileal contents, eventually supplemented with free amino acids, on the other hand, 20-30% of the amino acids were degraded by the flora either by deamination, with formation of ammonia, or by decarboxylation with formation of amines (histamine, putrescine, cadaverine, tyramine, phenylethylamine), Cadaverine, the decarboxylation product of lysine, was the most important amine detected. Both processes were severely decreased when the antibacterials Virginiamycin (50 ppm), Spiramycin (50 ppm), Carbadox (50 ppm) and copper sulphate (200 ppm) were present during the incubation of the ileal contents. The fermentation of caecal contents was characterized by a pronounced production of ammonia, slightly inhibited by Virginiamycin and Spiramycin. From tests of the amino acid decarboxylation activity of the dominant flora (E. coli, S. faecalis, L. acidophilus and L. fermenti) in the small intestine of the pig, it was found that E. coli is the main producer of amines in the small intestine. It is

concluded that the gut flora of pigs has a measurable negative influence on protein digestion in the small intestine and that this effect can be overcome by giving nutritional antibacterials.

**Dierick, N., Vandekerckhove, P. and Demeyer, D. 1974.** Changes in nonprotein nitrogen compounds during dry sausage ripening. *J. Food Sci.* 39: 301-304.

Concentration changes for NH3, total and individual free amino acids, total peptides, nucleotides, nucleosides and some individual amines were followed during ripening of dry sausage, with and without 'starter culture'. A decrease was observed for peptides, nucleotides, glutamic acid, histidine, tyrosine and ornithine, an increase for all other compounds, being most intense for total free amino acids during the first days of ripening. The rate of free amino acid production exceeded the rate of NH3 production. The presence of a starter culture intensified free amino acid production and peptide disappearance. A tenfold increase in the concn. of histamine, tyramine and putrescine was observed in the presence of a starter culture.

**Drjuchenko, E. A., and M. N. Kulikova. 1984.** Cadaverine and its possible role in host-parasite relationships in ascaridiasis. *Parazitologiya* 18 (4): 291-295.

The activity of lysine decarboxylase in Ascardia galli and in the liver and intestine of chickens was studied with the aim of assessing the formation of cadaverine. In homogenates of nematodes from naturally infected chickens, the activity of lysine decarboxylase was 60 ± 23 nM/mg protein, peaking at pH 4.4 and 5.6. The activity of the enzyme was found to be age- dependent, it declined from 379 nM/mg protein in young (38- to 40-day-old) nematodes obtained experimentally to 150 nM/mg in 50- to 55-day-old nematodes, and was absent in older A. galli. Higher activity was recorded in the nematode intestinal tissue than in the genital organs or body wall. In chicken intestine and liver homogenates the enzyme activity was 138 and 85 nM/mg and the pH optima were 4.4 and 5.6 respectively. It is suggested that cadaverine released by A. galli into the host intestine may, if the host defence reactions are low, impair the permeability of the intestines and facilitate the penetration of A. galli toxins.

**Dryuchenko, E. A., and L. S. Golikova. 1986.** Effect of Ascaridia infection on the formation of cadaverine in the tissues of chickens. *Trudy-Gel'mintologicheskoi-Laboratorii-Voprosy- biotsenologii-gel'mintov. 34: 30-33.*

Chickens aged 18 to 20 days were each infected with 200 Ascaridia ova and were examined after 2 h and on day 2 pi (tissue phase of infection) and on days 50 to 55 (mature nematodes in intestinal lumen) for lysine decarboxylase (LC) activity (which determines cadaverine formation) in the intestinal tissues and liver. LC activity was 2 to 3 times higher in the intestine of infected chickens than in controls and was 8.38, 47.75 and 34.0 nM/mg protein at 2 h, day 2 and day 50, resp. LC activity in the liver of infected birds showed a tendency to increase in all 8 experimental groups but was statistically significant only in 4. The increased production of cadaverine in the intestinal tissues as a factor of intestinal pathology is discussed.

**Dryuchenko, E. A., N. A. Eranova, and Z. K. Leutskaya. 1988.** On the role of cadaverine in immunological reactions during helminthiases. *Trudy-Gel'mintologicheskoi-Laboratorii. 36: 63-65.*

Leghorn chickens were immunized with increasing doses of Ascaridia antigen (AAg) or with tobacco mosaic virus (TMV) for one month. Lysine decarboxylase activity (LDA) (expressed in amounts of cadaverine) in intestinal and liver tissues increased after immunization. In chickens reimmunized 2 months after immunization, maximum LDA was observed on day 7; it declined by day 14 (i.e. when a peak amount of antibody was present). LDA changes were more marked in the liver of chickens immunized with AAg than in those immunized with TMV. A study of total serum protein, immunoglobulins and specific antibodies

showed that increases in LDA coincided with increased immunological activity. Increase of enzyme activity in the liver was more marked when AAg was used and correlated with increased (in comparison to TMV) total serum protein synthesis. Increased LDA in the intestine was in inverse correlation with the production of specific antibodies.

**Dufour, C., Dandrifosse, G., Forget, P., Vermesse, F., Romain, N. and Lepoint, P. 1988.** Spermine and spermidine induce intestinal maturation in the rat. *Gastroenterology* 95: 112-116.

Groups of 5 rats were given saline, spermidine (10 mumol daily), or spermine (6 mumol daily) by mouth on days 12, 13 and 14 after birth. They were killed on day 15. After the small intestine was removed, a 1-cm distal ileal segment was removed for histological examination and the remaining small bowel tissue was homogenized for further biochemical analysis. Polyamine induced structural and biochemical mucosal changes characteristic of postnatal maturation. Lactase, sucrase and maltase specific activities (mumol substrate hydrolysed per min g protein) were 80 ± 10, 10 ± 3 and 116 ± 19 for the saline-treated rats; 51 ± 7, 34 ± 2 and 315 ± 37 for the spermidine-treated rats; 25 ± 2, 46 ± 5 and 419 ± 63 for the spermine-treated rats, respectively. Similar results were obtained with rats, first treated with spermine (6 mumol) on day 7 of life, receiving spermine (6 mumol) daily as described above and killed on day 10. Dose-response experiments made as reported above in rats with treatment starting on day 12 showed that the maturational effects of spermine were dose-dependent.

**Duncan, M. W., G. A. Smythe, M. V. Nicholson, and P. S. Clezy. 1984.** Comparison of high-performance liquid chromatography with electrochemical detection and gas chromatography-mass fragmentography for the assay of salsolinol, dopamine and dopamine metabolites in food and beverage samples. *Journal of Chromatography, Biomedical Applications* 336 (1): 199-209.

HPLC with electrochemical detection and combined GLC-MS in the single-ion monitoring (SIM) mode were used to determine salsolinol, dopamine, 3,4-dihydroxyphenylacetic acid, 3,4- dihydroxyphenylethanol and norepinephrine in banana pulp, beer and soy sauce. The unique specificity of the SIM mode permits a simple 1-step extraction to be used even for complex sample matrices. Direct comparison of the chromatographic data obtained indicated that GLC-MS has quantitative and qualitative advantages over HPLC. The specificity of SIM and the benefits offered by incorporation of deuterated internal standards make GLC-MS the method of choice for valid identification and precise quantitation of these biogenic amines and their derivatives in complex matrices. The max. time required for any GLC-MS run was 5 min.

**Eckel, B., F. X. Roth, M. Kirchgessner, and U. Eidelsburger. 1992.** Influence of formic acid on concentrations of ammonia and biogenic amines in the gastrointestinal tract. 4. Investigations about the nutritive efficacy of organic acids in the rearing of piglets. *Journal of Animal Physiology and Animal Nutrition* 67 (4): 198-205.

For 41 days 45 weaned German Landrace X Pietrain pigs, 4 weeks old, were fed on diets supplemented with 0, 0.6, 1.2, 1.8 or 2.4% formic acid. Ammonia content in the stomach was significantly reduced by formic acid. In the small intestine and colon ammonia concentrations were decreased by 0.6 and 1.2% formic acid. In the caecum formic acid had no influence on ammonia. Concentrations of biogenic amines showed a large variation and in the small intestine were not changed by formic acid. Cadaverine, putrescine and spermidine in the caecum were reduced by formic acid. Spermidine concentration was decreased significantly by 2.4% formic acid. Spermine concentrations in the caecum were very low.

**Edmunds, W.J. and Eitenmiller, R.R. 1976.** Effect of storage time and temperature on histamine content and

histidine decarboxylase activity of aquatic species. *J. Food Sci.* 40: 516-519.

At ambient temp. (i) had significantly (P less than 0.05) more histamine than all species except (ii) and significantly (P less than 0.05) higher enzyme activity than all other species. All species showed significant (P less than 0.05) increases in histamine content with time. (i), (iii) and (v) showed significant (P less than 0.05) increases in enzyme activity with time while (ii) and (iv) showed no significant (P less than 0.05) changes. Highest level of histamine was 333 mug/g muscle in (i) and highest enzyme activity, also in (i), was 135.6 nmoles/min/g muscle. Even though measurable enzyme activity occurs in (ii), (iii), (iv) and (v), there is little chance that they would develop sufficient histamine to lead to intoxication. (i) could be capable of developing sufficient histamine to produce intoxication although advanced spoilage would probably be necessary to reach this stage.

**Edwards, R. A., R. H. Dainty, and C. W. Hibbard. 1985.** Putrescine and cadaverine formation in vacuum packed beef. *Journal of Applied Bacteriology* 58 (1): 13-19.

Beef shoulder and chuck joints (1-2 kg) vacuum packed 6-7 days after slaughter in Cryovac BB1 were obtained from a commercial supplier (3 batches being studied) and stored at +1 degree C for up to 8 wk. Sterile control samples of semitendinosus muscle (approx. 1 kg) were packaged and stored similarly. At intervals, samples were assessed microbiologically (total count, and counts of lactic acid bacteria, Brochothrix thermosphacta, Pseudomonas spp., and Enterobacteriaceae), pH was measured, cadaverine and putrescine were determined, and odour was evaluated. Tables of results are given. The control sample remained essentially bacteria-free throughout storage; its pH increased slightly, and odour changed from meaty to sour, acid. Putrescine content remained within the range 0.8-1 mug/g; cadaverine content was 0.1-0.3%. In the commercial samples, total count reached O6.5 x 10-7/cm-2, the microflora of most being dominated by lactic acid bacteria. All stored, commercial samples had low counts of pesudomonads, B. thermosphacta and Enterobacteriaceae. The pH varied irregularly during storage. Typical vacuum-packaged meat odours were observed in greater than 60% of samples; transient H2S-like odours were observed in some samples, and more persistent 'pickles', 'sweet' or 'faecal' odours occurred in some samples stored for P7 wk. Odour did not appear to be closely related to microflora. Diamine contents increased during storage, concn. of O110 mug putrescine/g and O158 mug cadaverine/ g being observed. Diamine concn. were not closely correlated with storage time; moderate correlations with bacterial counts were observed.

**Evans, P.T. and Malmberg, R.L. 1989.** Do polyamines have roles in plant development? *Ann. Rev. Plant Physiol. Plant Molecul. Biol.* 40: 235-269.

Evidence for polyamine involvement in cell division, embryogenesis, rooting, flowering, fruit development, pollen tube growth and senescence, postulated links with growth regulators especially ethylene and gibberellic acid and the role of polyamines in plant response to a variety of stresses are reviewed.

**Ferencik M. 1970.** Formation of histamine during bacterial decarboxylation of histidine in the flesh of some marine fishes. *J. Hyg. Epidemiol. Immunol.* 14: 52-60.

**Galston, A. W. 1983.** Polyamines as modulators of plant development. *Biosci.* 33: 382-386.

**Eitenmiller, R. R., P. E. Koehler, and J. O. Reagan. 1978.** Tyramine in fermented sausages: factors affecting formation of tyramine and tyrosine decarboxylase. *Journal of Food Science* 43 (3): 689-693.

Factors influencing tyramine formation and the presence of tyrosine decarboxylase activity in natural microflora and starter (Pediococcus cerevisiae) fermented sausages were determined. Max. levels of tyrosine decarboxylase were found during the period of rapid acid development in the natural microflora fermentations. Higher levels of decarboxylase were present in the natural fermented sausages than the starter fermented sausages. Tyrosine decarboxylase activity increased during the drying treatment in the natural microflora sausages but remained low in the starter fermented sausages. Greatest tyramine contents ( greater than 280 mug/g) were found in natural microflora sausages that had free L -tyrosine (500 mug/g) added prior to fermentation. Tyramine contents of natural microflora fermented sausages without added L -tyrosine and of starter fermented sausages were generally similar (approx. 80 mug/g). In the starter fermented sausages, tyramine and tyrosine decarboxylase development comparable to the amounts found in natural microflora fermented sausages (containing added L -tyrosine) occurred only in a sausage that contained added L -tyrosine that was inoculated with a tyrosine decarboxylase-positive Streptococcus faecalis. The study indicated that the natural meat microflora developed during the ageing of salted meat can develop tyrosine decarboxylase activity necessary for rapid conversion of tyrosine to tyramine. The use of a starter culture such as P. cerevisiae appears to decrease the possibility of development of a microflora during fermentation that would possess both tyrosine decarboxylase activity and proteolytic activity necessary to produce fermented sausages with potentially hazardous tyramine concn.

**Elfberg, E. J., and U. Edberg. 1991.** Histamine and tyramine in cheeses available on the Swedish market. *Var Foda* 43 (4-5): 228-235.

An HPLC method was used to determine the histamine and tyramine concn. of approx. 40 cheese varieties (including (i) Swedish cheeses and (ii) cheeses of foreign origin available on the Swedish market). For some varieties both 'mild' and 'mature' cheeses were analysed. Tables show histamine and tyramine concn. for (i) and (ii) cheeses. Cheese varieties are also grouped according to whether they contain a high, medium or low concn. of histamine (>150, 10-150 or <10 mg/kg resp.), or a high, medium or low concn. of tyramine (>300, 10-300 or <10 mg/kg resp.) Highest concn. of histamine was found in Cheddar, Wasterbotten and Prastost cheeses, and highest concn. of tyramine was found in Cheddar, 'extra-mature' Greve and Ostgota starkost cheeses. Cheese varieties with low concn. of both histamine and tyramine included Drabant, Edam, 'mild' Greve, Graddost, 'mild' Herrgard, Hushallsost and Svecia.

**Elia, M. 1992.** Glutamine in parenteral nutrition. *International Journal of Food Sciences and Nutrition* 43 (1): 47-59.

This brief review examines the developments which have been responsible for changing the form in which substrates have been infused.

**Etter, R., S. Dietrich, and R. Battaglia. 1990.** Estimation of biogenic amines in foods. *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene* 81 (1): 106-119.

A simple method for the estimation of the most important biogenic amines is presented. The amines are extracted from solid food samples with a 1:1 mixture of 1 N perchloric acid and acetonitrile and converted to their dansyl derivatives. The derivatives were separated by reversed-phase high-performance liquid chromatography using ultraviolet and fluorescence detectors. Quantitation was achieved with the internal standard diaminoheptane. The estimation limit is about 0.5 mg/litre for liquid food samples and 5 mg/kg for solid food samples. Recoveries are typically 80 to 110%. Results are presented for Tilsit cheese, red wine and dried meat.

**Fabian, V., M. Pinter Szakacs, and I. Molnar Perl. 1990.** Gas chromatography of tryptophan together with other

amino acids in hydrochloric acid hydrolysates. *Journal-of-Chromatography 520: 193-199.*

Hydrolysis of proteins with hydrochloric acid using tryptamine [3-(2-aminoethyl)indole] as additive allowed measurement of tryptophan without destruction together with other amino acids by gas chromatography. An extensive study established optimum conditions for protein hydrolysis (time and temperature of hydrolysis, amount of tryptamine) and for derivatization of amino acids. Amino acid contents (including tryptophan) of standard proteins such as lysozyme, bovine and human albumin, human gamma- globulin, casein and alpha-chymotrypsin and protein matrices (meat and fish meals, sunflower) were estimated, after hydrochloric acid hydrolysis (4 h, 145°C) in the presence of tryptamine, as N,O,(S)- trifluoroacetyl isobutyl esters with SE- 30 as the stationary phase. The reproducibility of the measurements was 4.6% (relative standard deviation) or less.

**Fairgrieve, W. T., M. S. Myers, R. W. Hardy, and F. M. Dong. 1994.** Gastric abnormalities in rainbow trout (Oncorhynchus mykiss) fed amine-supplemented diets or chicken gizzard-erosion- positive fish meal. *Aquaculture* 127 (2/3): 219-232.

Fish meals produced from fish containing high levels of histamine can be acutely toxic to chickens, causing gizzard erosion (GE), black vomit disease, and death after 3-5 days' feeding. Fish meals are sometimes selected for aquaculture use on the basis of chicken toxicity testing, although data supporting this practice are scarce. In this study, growth, feed intake and development of gastric abnormalities were assessed in juvenile rainbow trout, 5.5 g, given diets with fish meal acutely toxic to chickens, or casein and fish meal diets supplemented with histamine and 2 suspected potentiators of histamine toxicity, putrescine and cadaverine, and excessively heated. No signs of acute toxicity or mortality occurred in the fish during the 16- week study. Fish given diets with GE-positive fish meal had distended stomachs, but no gastric lesions or cellular abnormalities. Similar effects were obtained by feeding diets containing casein or GE-negative fish meal supplemented with histamine (2000 mg/kg dry diet). Addition of putrescine and cadaverine (500 mg/kg dry diet each) to histamine-supplemented diets had no further effect. Feed intake, feed conversion efficiency and growth were similar among dietary treatments, indicating that stomach distension did not reduce feed intake or impair gastric function. It was concluded that rainbow trout are less sensitive than chickens to GE-positive fish meal and that there is no relation between positive GE score and nutritional value of fish meal for rainbow trout. This study also showed that stomach distension resulting from feeding diets with GE- positive fish meal could be duplicated by feeding diets supplemented with histamine 2000 mg/kg diet.

**Fernandez Salguero, J., and I. M. Mackie. 1987.** Comparative rates of spoilage of fillets and whole fish during storage of haddock (Melanogrammus aeglefinus) and herring (Clupea harengus) as determined by the formation of non-volatile and volatile amines. *International Journal of Food Science & Technology* 22 (4): 385-390.

The concn. of nonvolatile and volatile amines formed in herring and haddock during storage as fillets and as whole fish, in ice and at 5 degree C, were determined. Comparison of the rates of formation of the major nonvolatile amines (histamine, cadaverine and putrescine) and trimethylamine showed that haddock fillets deteriorated more rapidly than whole gutted fish and that ungutted herring spoiled more rapidly than fillets. The value of amines as indices of spoilage in fish is discussed.

**Fernandez Salguero, J., and I. M. Mackie.** 1979. Histidine metabolism in mackerel (Scomber scombrus). Studies on histidine decarboxylase activity and histamine formation during storage of flesh and liver under sterile and non-sterile conditions. *Journal of Food Technology* 14 (2): 131-139.

The concn. of histidine and histamine in the flesh and liver of mackerel were determined during storage at 0, 2, 10 and 23 degree C. Under sterile and non-sterile conditions little histamine was produced during storage of muscle even after 18 days at 0 degree C. At 10 degree C the levels of histamine exceeded 100

mg/100 g tissue in both liver and muscle after 5 days' storage. The concn. of histidine in muscle remained virtually constant throughout all storage periods but in liver it increased markedly. Histamine showed greatest increase in liver samples. Histidine decarboxylase activity as measured by release of -1-4CO2 from -1- 4C-L -Histidine in crude extracts showed a decrease in muscle and an increase in liver. The significance of bacterial and tissue enzymes on the production of histamine is discussed.

**Fonberg Broczek, M., B. Windyga, J. Kozlowski, D. Sawilska Rautenstrauch, and S. Kahl. 1988.** Estimation of histamine in fish preserves by a spectrofluorimetric method. *Roczniki Panstwowego Zakladu Higieny* 39 (3): 226-230.

The content of histamine in a total of 30 samples of various fish preserves was estimated by the spectrofluorimetric method recommended by AOAC. The detection limit was 1 mg/100 g. Values obtained varied from below 1 to 110.1 mg/100 g. In only 4 samples of sardines in oil imported from Yugoslavia was the histamine content above the limit of tolerance accepted in Poland, i.e., 20 mg/100 g. It was concluded that the procedure applied is rapid, sensitive and supplies reproducible results and that it may be used as a reference method for estimating histamine in fish preserves.

**Foo, L. Y. 1976.** Scombroid poisoning. Isolation and identification of 'saurine'. Journal of the Science of Food and Agriculture; 27 (9): 807-810.

Samples of fish which had been involved in allergy-like food poisoning incidents [smoked fillets of Kahawai (Arripis trutta) and canned tuna (Euthynnus alletteratus)] were examined for the presence of 'saurine', a toxin reported to be the causative agent in scombroid poisoning. Chromatograms of extracts developed in acidic solvents showed 2 distinct areas which possessed vagal stimulating activity with guinea pig ileum. Both these areas contained histamine and this suggests that 'saurine' is a histamine salt.

**Frank, H. A., and Yoshinaga D.H. 1984.** Histamine formation in tuna. *ACS Symposium Series* 262: 443-451.

Spoilage of skipjack tuna was studied under controlled incubation conditions, using histamine formation as an indicator of decomposition. The bacteria in skipjack loin tissue were enumerated during incubation at 38 degree C, the optimum decomposition temp., and representative spoilage organisms were identified. Histamine-forming isolates included 4 Gram-negative, facultatively anaerobic bacteria (Enterobacter aerogenes, Klebsiella pneumoniae, Proteus mirabilis, and Vibrio alginolyticus) and a single anaerobic sporeformer, Clostridium perfringens. Several isolates were used to determine if histamine-forming bacteria could grow and if whole cell suspensions could decarboxylate histidine at low temp. At 4 degree C K. pneumoniae grew in skipjack infusion broth and its resting cells produced histamine from histidine. Although C. perfringens strains did not grow at low temp., cells grown at 38 degree C were able to produce histamine at 4 degree C. [See FSTA (1986) 18 2R6.].

**Friedman, M. 1993.** Chemical and biochemical basis for beneficial effects of sulphydryl compounds on food safety. *Pages 193-197 in Food and Cancer Prevention: Chemical and Biological Aspects [edited by Waldron, K. W.; Johnson, I. T.; Fenwick, G. R.]. Royal Society of Chemistry (RSC), Cambridge, UK.*

Some approaches towards reducing the deleterious effects of representative food toxicants, based on the reactivity of the sulphydryl group with electrophilic centres, are reviewed. These include use of the double bond of dehydroalanine to prevent lysinoalanine formation, the double bond of furan rings of aflatoxins to suppress mutagenicity, and disulphide bonds of plant protease inhibitors to reduce potential carcinogenicity. It is concluded that the antioxidant and antitoxic effects of SH- containing amino acids, peptides and proteins are due to their abilities to act as reducing agents, scavengers of oxygen radicals, strong nucleophiles that

can trap electrophilic compounds, precursors of cellular glutathione and inducers of cellular detoxification.

**Friedman, M., and P. A. Finot. 1991.** Improvement in the nutritional quality of bread. *Nutritional and Toxicological Consequences of Food Processing (edited by Friedman, M.). Advances-in-Experimental-Medicine-and-Biology. 289: 415- 445.*

To assess whether the dipeptide N-epsilon-(gamma-L-glutamyl)-L- lysine (glutamyl-lysine) can serve as a nutritional source of lysine, the growth of Swiss Webster mice fed on (a) an amino acid diet in which lysine was replaced by 6 dietary levels of glutamyl-lysine; (b) wheat gluten diets fortified with lysine; (c) a wheat bread-based diet (10% protein) supplemented before feeding with lysine or glutamyl-lysine (0, 0.75, 1.50, 2.25 and 3% lysine HCl-equivalent in the final diet), not co-baked and (d) bread diets co-baked with these levels of lysine or glutamyl- lysine was evaluated. With the amino acid diet, the relative growth response to glutamyl-lysine was about half that of lysine. The effect of added lysine on the nutritional improvement of wheat gluten depended on both lysine and gluten concentrations in the diet. With 10 and 15% gluten, 0.37% lysine HCl produced an increase in weight gain. Further increase in lysine HCl to 0.75% proved detrimental to weight gain. Lysine HCl addition improved growth at 20 and 25% gluten in the diet and did not prove detrimental at 0.75%. For whole bread, glutamyl-lysine served nearly as well as lysine to improve weight gain. The nutritive value of bread crust fortified or not was less than that of crumb or whole bread. Other data showed that lysine or glutamyl-lysine at the highest level of fortification, 0.3%, improved the protein quality (PER) of crumb over that of crust or whole bread, indicating a possible greater availability of the second- limiting amino acid, threonine, in crumb. These data and additional metabolic studies with U-14-C glutamyl-lysine suggest that glutamyl-lysine, co-baked or not, is digested in the kidneys and utilized in vivo as a source of lysine.

**Fukuhara, K., Y. Ishigami, R. Katsumura, T. Ito, Y. Matsuki, and T. Nambara. 1982.** [Determination of tyramine in foods by high performance liquid chromatography with fluorescence detection.]. *Journal of the Food Hygienic Society of Japan [Shokuhin Eiseigaku Zasshi]* 23 (5): 384-387.

A sensitive and simple detn. of tyramine in food by HPLC without derivatization is described. The clean-up of tyramine was efficiently attained by extraction with 0.2% trichloroacetic acid in the presence of chloroform. Tyramine was separated on a reversed-phase HPLC column (fluorescence detection) with 0.2% ammonium carbonate and methanol (1:3) as the mobile phase. The quantitation limit of tyramine was 8 ng and recoveries of tyramine added to foods were 86-100%. Amounts of tyramine in several foods were determined by the proposed method [salami and wiener sausage, bacon, smoked cheese, processed cheese, natural cheese, miso, soy sauce].

**Furst, P., S. Albers, and P. Stehle. 1990.** Glutamine- containing dipeptides in parenteral nutrition. *Journal of Parenteral and Enteral Nutrition* 14 (4): Supplement, 118S-124S.

Of the total pool of muscle free intracellular amino acids, glutamine represents about 60%. During catabolic stress, a marked reduction (50%) of this pool occurs; the depletion is not reversible by therapeutic efforts or conventional nutritional means. If maintenance of the intracellular glutamine pool promotes conservation of muscle protein, there is a theoretical case for use of glutamine supplements in the parenteral nutrition of patients with injury and infection. Glutamine is too unstable and poorly soluble for addition to existing preparation in its native form, but this drawback can be overcome by the use of synthetic stable and highly soluble glutamine-containing dipeptides. Studies in vivo in man and animals provide firm evidence that a synthetic glutamine-containing dipeptide, L- alanyl-L-glutamine (Ala-Gln), readily hydrolysed after its intravenous administration. The results also indicate a safe and efficient use of Ala-Gln as a source of free glutamine in parenteral nutrition. In clinical studies, nitrogen balance was more positive in catabolic patients receiving a peptide- supplemented solution than in control patients given isonitrogenous, isoenergetic total parenteral nutrition. Muscle glutamine concentrations were markedly decreased in the control groups. The

intracellular concentrations were not influenced after severe injury, but were maintained in postoperative trauma. It is inferred that the increased intestinal requirement and cellular demand for metabolic fuel during catabolic stress is matched by an increased demand on muscle glutamine, resulting in intracellular glutamine depletion. Thus the delivery of adequate amounts of glutamine is essential to maintain the integrity of intestinal mucosa and rapidly proliferating cells, to preserve the muscle glutamine pool, and to improve overall N economy in conditions of stress. .

**Gajewska, R., E. Lipka, and Z. Ganowiak. 1991.** Levels of histamine and tyramine in selected food products. *Roczniki Panstwowego Zakladu Higieny* 42 (1): 1-7.

A total 600 samples of food products were taken from processing plants and shops in North Poland between 1987 and 1988. The contents of histamine and tyramine were estimated. Values for histamine in fresh, smoked or processed fish ranged from 0 to 8.0, from 0 to 11.0 and from 0 to 16.0 mg/100 g, respectively. Corresponding values for tyramine were from 0 to 2.6, from 0 to 6.0 amd from 0 to 8.0 mg/100 g. Histamine and tyramine in 4 types of fish preserves were from 1.3 to 13.5 and from 1.0 to 10.0 mg/100 g, respectively. The histamine content in hard, soft or processed cheeses was from 1.0 to 10.8, from 0 to 7.0 and from 1.8 to 7.0 mg/100 g, respectively, corresponding values for tyramine being from 1.3 to 20.0, from 2.5 to 10.0 and from 2.0 to 8.0 mg/100 g. The level of histamine in samples of tomato paste, yeasts, wine, fresh cabbage and sauerkraut varied from 2.0 to 16.6, from 0.5 to 4.4, from 3.0 to 6.6, from 0 to 3.0 and from 0.6 to 11.0 mg/100 g, respectively. Corresponding values for tyramine ranged from 0.5 to 6.0, from 0 to 5.0, from 1.0 to 3.3, from 0 to 4.0 and from 1.0 to 8.0 mg/100 g. It was concluded that the contents of histamine and tyramine in all products under examination were low.

**Ganowiak, Z., R. Gajewska, and E. Lipka. 1990.** Effect of technological processes on the histamine content of processed fish. *Roczniki Panstwowego Zakladu Higieny* 41 (3-4): 180-186.

The histamine content of fish was estimated at different stages of manufacture for 4 types of fish preserves; mackerel in oil, mackerel in tomato sauce, hake fillets and a preserve made from southern blue whiting. In addition changes in the histamine content of pickled herring and smoked herring during the manufacturing processes were studied. Canning had no effect on the histamine content of fish. Pickling and hot smoking increased the histamine content of herring (pickling: 3.60 to 11.30; hot smoking 1.00 to 3.50 mg/100 g, respectively). All values were below the limit of tolerance i.e. 20 mg/100 g. It is concluded that the manufacturing processes if correctly applied, do not increase the histamine content of fish.

**Garcia Moreno, C., J. C. Rivas Gonzalo, M. J. Pena Egido, and A. Marine Font. 1983.** Improved method for determination and identification of serotonin in foods. *Journal of the Association of Official Analytical Chemists* 66 (1): 115-117.

A previously described method [see FSTA (1980) 12 9J1187] to identify and quantitate serotonin in foods was improved. The extraction and separation of serotonin from interfering substances was improved, and the scope of material to which the method may be applied was widened. The relative s.d. (RSD) for repeated detn. of serotonin in canned fried tomato puree and the average recovery of serotonin added to the same sample was 6.25 and 89.9%, resp. The method showed the presence of serotonin in apricots, cherries, and peaches.

**Garcia Moreno, C., A. Nogales Alarcon, A. Gomez Cerro, and A. Marine Font. 1980.** Spectrofluorometric determination and thin layer chromatographic identification of serotonin in foods. *Journal of the Association of Official Analytical Chemists* 63 (1): 19-21.

A method is described for determining serotonin in foods, based on alkaline butanol/sand column elution followed by spectrofluorometric detn. with TLC confirmation. The method was applied to fresh bananas, banana-based baby foods, and fresh and canned tomatoes. Average recovery was 91%. Amounts of serotonin found were 10-30 p.p.m. in bananas, 0.1-1.9 p.p.m. in banana- based baby foods, 4.4-5.6 p.p.m. in fresh tomatoes, and 2.8-5.6 p.p.m. in canned tomatoes.

**Gasco, L., and R. Barrera. 1972.** The use of derivatives for the gas-chromatographic identification of alcohols, primary and secondary amines, and thiols in food aromas. *Analytica Chimica Acta* 61 (2): 253-264.

Conditions are given for the formation, from volatile concentrates of food aromas, of the following derivatives: alkyl- and thioalkylbenzoates for alcohols and thiols, N- alkylbenzamides for primary and secondary amines, and 2,4- dinitrophenylalkylthioethers and 2,4-dinitrophenylalkylsulphones for thiols. Gas-chromatographic separations were carried out on 5% SE-30 silicone gum rubber on Chromosorb G-HP in 1-m columns at temp. ranging from 125 to 185 degree . Correlations between retention data and molecular structure are studied and some typical chromatograms (of alkylbenzoates of alcohols from apple juice; of benzamides of amines from irradiated hake, and of 2,4- dinitrophenylalkylthioethers from irradiated hake) are given. The chromatographic information from these derivatives is a significant help for the identification of some volatile constituents of food aromas.

**Gehrke C. W., Kuo, K. C., Ellis, R. L. and Waalkes, T P. 1977?** Poly amines an improved automated ion exchange method. *J. Chromatog.* 143: 345-361.

An accurate, precise and improved automated cation-exchange chromatographic method with ninhydrin detection for the analysis of di- and polyamines (putrescine, cadaverine, spermidine and spermine) was developed. Different types of biological fluids e.g., urine, blood plasma, blood sera, tissue extracts and cancer cell culture media can be analyzed under identical chromatographic conditions. The simplicity and precision of the method was achieved by eliminating the sample preseparation and using an internal standard technique. Not only was the sample preparation simplified, but the accuracy and precision and sensitivity of the method were greatly improved, with 24 unattended analyses performed each day. With minor modifications of the instrument a 2-fold analytical output can be achieved with analysis time cut to 30 min. More than 2000 urine and hundreds of other physiological samples were analyzed over 6 mo. by this method with a relative SD from 3.3-7.8% and recoveries of 94-97%.

**Golovnya, R. V. 1982.** Some analytical problems in flavour research. *Journal of Chromatography, Chromatographic Reviews* 251 (3): 249-264.

Problems of studying aroma volatiles in foods are discussed relative to extraction and concentration, GC separation of the mixtures, and identification of the individual components. The technique of standardless GC identification of odour components developed on the basis of Kovats retention indices is recommended and has been applied extensively. More than 70 organic bases (including secondary aliphatic amines and heterocyclic compounds) have been found in foods, and possible amine precursors of carcinogenic N-nitrosamines have been identified. Results are tabulated for computer-aided identification of amines in boiled beef, salmon, bread, Chanakh cheese, casein, and coprecipitate. Studies on the unpleasant odour from stored casein and coprecipitate showed that amines, not carbonyl compounds, were responsible. A correlation was established between the accumulation of amines and a deterioration in the nutritional value of casein and coprecipitate (84% and 57% resp. after 1 yr storage). A comparison of volatile compounds from boiled meat and Maillard reaction products showed that processes occurring during the Maillard reaction are not identical to those occurring during boiling or frying of foods.

**Grant, A. L., Holland, R. E., Thomas, J. W., King, K. J. and Liesman, J. S. 1973.** Effects of dietary amines on the small intestine in calves fed soybean protein. *J. Nutr.* 119: 1034-1041.

An experiment was conducted using 16 Holstein male calves from 4 to 21 d of age to compare 1) the effects of an all-milk protein milk replacer (MPR) and a milk replacer with 20% of the protein from soy protein concentrate (SPC) on morphological and enzymic small intestinal variables, and 2) the effects of SPC plus putrescine (SPP) or SPC plus ethylamine (SPE) on intestinal variables. Small intestinal absorption, based on xylose absorption tests, was greater in calves fed MPR than in those fed SPC ($P < 0.01$) and was intermediate in SPP. and SPE-fed calves. Small intestinal segments were surgically excised from the proximal and distal jejunum of all calves at 7, 14 and 21 d of age. Villus length tended to be greatest in calves fed MPR, and mitotic index was least in SPC-fed calves ($P < 0.05$). Mucosal protein concentration was 46, 41, 44 and 44 .mu.g/mg mucosa for calves fed MPR, SPC, SPP and SPE, respectively. The ratio of mucosal protein:RNA was greatest in calves fed MPR, least in those fed SPC at d 7 ($P < 0.01$) and d 14 ($P < 0.05$), and intermediate in calves fed SPP and SPE. In proximal jejunum, activity of mucosal ornithine decarboxylase (ODC, EC 4.1.1.17; the rate-limiting enzyme in polyamine biosynthesis) in calves fed SPP was less than 50% of that in calves fed MPR, SPC or SPE. The activity of lactase (EC 3.2.1.108) and ODC in distal jejunum was 50% less in calves fed soybean protein than in those fed MPR. We conclude that soy protein concentrate reduces small intestinal absorption and enterocyte proliferation in preruminant calves. Furthermore, putrescine and ethylamine, when added to soybean protein diets, enhance enterocyte proliferation and partially prevent reductions in absorption.

**Grant, A. L., J. W. Thomas, K. J. King, and J. S. Liesman. 1990.** Effects of dietary amines on small intestinal variables in neonatal pigs fed soy protein isolate. *Journal of Animal Science* 68 (2): 363-371.

Six litters of newborn crossbred piglets were given a 100%-milk protein milk substitute (MPMS), or a milk substitute with 20% of milk protein replaced with a soya protein isolate without (SPMS) or with putrescine dihydrochloride 25 g/kg diet or ethylamine hydrochloride 25 g/kg diet. Small intestinal xylose absorption increased from 1 to 2 weeks old in pigs fed on MPMS, putrescine and ethylamine supplemented diets, but not in pigs fed on SPMS. Crypt depth in pigs fed on the milk-soya diet was lower (9.4%) than the crypt depth in pigs fed on the other diets, but mitotic index was not different among diets. Mucosal protein, DNA and RNA concentrations and mucosal brush border sucrase and cytosolic dipeptidase activities were least in pigs fed on putrescine and ethylamine supplemented diets. Concentration of mucosal putrescine was greatest ($P<0.002$) in the distal regions of the small intestine of pigs fed on putrescine. Mucosal ornithine decarboxylase activity was inhibited by putrescine ($P<0.02$), but was not affected by the soyabean protein isolate. Results suggest that supplementing soya protein isolate diets with amines may increase intestinal absorption and enterocyte proliferation.

**Grant, A. L., and J. W. Thomas. 1987.** Improving milk replacers containing soybean proteins with dietary amines. *Research-Report,-Agricultural-Experiment-Station,-University-of- Michigan. 1987, No. 487, 40-42.*

When given to piglets milk replacer containing milk protein 80 and a special soyabean protein 20% permitted normal growth and microscopic structure in the small intestine but xylose absorption at 2 weeks old was less than with 100% milk protein in the replacer. With putrescine dihydrochloride or ethylamine hydrochloride, 2.5% in replacer, xylose absorption was normal.

**Gray, J. I., and D. G. Roberts. 1970.** Retention and release of volatile food flavour compounds. *Journal of Food Technology* 5 (3): 231-39.

Factors controlling the adsorption and retention of volatile flavour compounds such as aldehydes, ketones,

alcohols, amines and sulphides from the various substrates occurring in foods, such as pectin, gelatin, alginate and silica gel, were investigated. Parameters such as (i) activation energy of desorption, (ii) the reaction order of the desorption process, and (iii) heats of preferential sorption were studied. Parameters (i) and (ii) were measured using a thermal balance and (iii) using a flow microcalorimeter. (i) was obtained for the release of ethylamine from pectin, but insufficient amine was adsorbed to obtain values for Schardinger-beta dextrin, gelatin and sodium alginate. All the flavour compounds studied gave detectable (iii) with silica gel. Of the other systems studied only trimethylamine and ethylamine gave detectable (iii) both with pectin and Schardinger-beta- dextrin.

**Greer, F., Brewer, A.C. and Pusztai, A. 1985.** Effect of kidney bean (Phaseolus vulgaris) toxin on tissue weight and composition and some metabolic functions of rats. *Br. J. Nutr.* 54: 95-103.

**Grillo, M. A., S. Colombatto, and D. C. Pezzali. 1987.** Effect of selenium on polyamine metabolism. *Italian Journal of Biochemistry* 36 (1): 29A-30A.

Because polyamines, like selenium, have a role in cell proliferation, studies were made of the relation between Se and polyamine metabolism using rats of 200 to 250 g and chickens 15 days old. In the acute studies they were given a single intraperitoneal injection of Na2SeO3 0.5 mg/100 g and were killed 6 h later. In a chronic feeding study, rats were fed for 5 weeks on a normal diet supplemented with selenite 5.25 mg/kg. Liver ornithine decarboxylase, S-adenosylmethionine decarboxylase, spermidine acetyltransferase and polyamines were estimated, plus bursa of Fabricius values in chickens. It is concluded that the modifications in the enzyme activities could not be due to a direct effect of selenite on the enzymes, as the increase in Se concentration was small, and of the same order of magnitude in both tissues.

**Grimble, G. K., and D. B. A. Silk. 1989.** Peptides in human nutrition. *Nutrition Research Reviews* 2: 87-108.

Advances in research on peptides and their role in clinical protein nutrition are discussed. The application of peptides to oral and intravenous nutrition is reviewed. Large-scale chemical synthesis of specific dipeptides and tripeptides is proposed as a solution to problems in parenteral nutrition. It is suggested that amino acid requirements in parenteral nutrition may be satisfied by infusion of mixtures of synthetic dipeptides or short-chain peptides.

**Gruger, E.H. Jr. 1972.** Chromatographic analyses of volatile amines in marine fish. *J. Agric. Food Chem.* 20: 781-785.

Amines, extracted from tissues of sablefish (*Anoplopoma fimbria*), coho salmon (*Oncorhynchus kisutch*), and salmon roe (genus, *Oncorhynchus*), were analysed by gas chromatography, and also by thin-layer chromatography-spectrophotofluorometry of the corresponding 5- dimethylamino-1-naphthalene-sulphonamides. The data provide evidence for the presence of dimethylamine in long-stored frozen salmon flesh and its absence in sablefish flesh above a detection limit of 7 ng/g wet tissue. The methylamines and diethylamine were the only amines confirmed at levels above the limits of detection.

**Haaland, H., M. Espe, L. R. Njaa, and H. Myklestad. 1990.** Chemical composition and variation in some parameters during storage of 8 formic acid silages prepared from capelin. *Fiskeridirektoratets Skrifter. Serie Ernaering* 3 (2): 59-74.

Fish silage may partly replace fish meal and fish oil in feeds for farmed fish and furbearing animals. Quality

of capelin silage, prepared from one batch of frozen capelin untreated or treated with potassium sorbate, ethoxyquin plus 2 levels of formic acid, or thawed and steamed and kept in plastic containers at 10°-12°C was sampled at ensiling and after 1, 1.5, 3, 6, 9 and 12 months' storage. Only total volatile nitrogen (TVN) and biogenic amines were suitable indicators of silage quality. Tyramine was the most promising, as it occurred in the silages in the same amounts as were present in the raw material. As TVN reached a plateau after about 3 months' storage, further methods for assessing the degree of autolysis in silages are needed.

**Haaland, H., and L. R. Njaa. 1990.** Fish silages prepared from raw material of varying quality; chemical analysis related to balance experiments in rats. *Fiskeridirektoratets Skrifter. Serie Ernaering* 3 (1): 27-35.

Capelin (Mallotus villosus) was stored for 1, 3 or 5 days and Norway pout (Boreogadus esmarkii) was stored for 1 day then both treated with formic acid 16 and 22 g/kg and stored for 1 or 7 days or 3 months. In rats fresh capelin and pout gave similar weight gains and nutrient digestibilities and utilization. Silages from capelin, which had been kept for 7 days, had similar effects as fresh capelin. Pout raw material produced more weight gain and greater protein utilization than did the corresponding silage. All silages stored for 3 months were less well utilized than those stored for 7 days. Silages, 7 days old, prepared from capelin stored for 5 days produced similar weight gains and digestibilities but were less utilized than silages from the fresh materials, due to high concentrations of volatile nitrogen compounds and reduced amounts of essential amino acids and biogenic amines.

**Haaland, H. and Njaa, L.R. 1989.** Effect of temperature on the autolysis of capelin silages stored for one year. *Fiskeridirektoratets Skrifter. Serie Ernaering* 2: 219-226.

Conventional formic acid silage was prepared from frozen capelin stored at pH 3.8 to 4.0 for 12 months at 2°, 20° and 37°C, during which period samples were chemically analysed at intervals. During storage soluble nitrogen rapidly attained a peak of 90% of total N at 20° and 37°; the increase was slower and the peak lower at 2°. Ninhydrin-reactive substances, ammonia and alpha-amino N, increased during the first months and stabilized at higher values at 20° and 37° than at 2°. There was no change in amino acid composition during storage. Total volatile N and ammonia N increased during the whole period, although faster during the first week. Amide N decreased at a corresponding rate. Ammonia N was below 3.5% of total N.

**Haaland, H., and L. R. Njaa. 1989.** Nitrogen balance and growth in young rats given the amines cadaverine, putrescine, histamine and tyramine in fish meal diets. *Fiskeridirektoratets Skrifter. Serie Ernaering* 2 (7): 213-218.

Cadaverine, putrescine, histamine and tyramine added in combination to fish meal diets, given to young rats, at 420, 220, 125 and 220, or at 1710, 910, 540 and 910 mg free bases/kg diet, respectively, had no adverse effects on growth or nitrogen utilization.

**Halasz, A., A. Barath, L. Simon Sarkadi, and W. Holzapfel. 1994.** Biogenic amines and their production by microorganisms in food. *Trends in Food Science and Technology* 5 (2): 42-49.

The production of biogenic amines (including putrescine, serotonin, cadaverine, histamine, spermine, spermidine and tyramine) in foods by microorganisms is reviewed. Topics discussed include: biogenic amine production by Lactobacillus, Salmonella, Clostridium, Escherichia coli, Bacillus, Enterococcus and Klebsiella spp.; and biogenic amine content of meat, fish, cheeses, sauerkraut, beer, fruit and vegetables.

**Halasz, A., A. Barath, L. Simon Sarkadi, and W. Holzapfel. 1994.** Biogenic amines and their production by microorganisms in food. *Trends in Food Science & Technology* 5 (2): 42-49.

Production of biogenic amines (including histamine, putrescine, cadaverine, spermine, spermidine, serotonin, tyramine) in foods by microorganisms is discussed. Aspects considered include: biogenic amine production by bacteria (Lactobacillus buchneri, Escherichia coli and Salmonella, Clostridium and Bacillus spp.); and biogenic amine content of foods (meat, fish, cheese, sauerkraut, beer, fruit and vegetables).

**Hamano, T., Y. Mitsuhashi, and Y. Matsuki. 1981.** Glass capillary gas chromatography of secondary amines in foods with flame photometric detection after derivatization with benzenesulfonyl chloride. *Agricultural and Biological Chemistry* 45 (10): 2237-2243.

A simple and selective gas chromatographic method was established for determining naturally occurring secondary amines. Secondary amines separated from foods by extraction with dichloromethane and reextraction with HCl were readily converted into the corresponding sulphonamides by reaction with benzenesulphonyl chloride under alkaline condition. Gas chromatography was carried out with a capillary coated with OV- 101 and a flame photometric detector. Column temp. was programmed from 170 to 230 degree C at a rate of 5 degree C/min. The obtained sulphonamides were separated from one another within 40 min. Eleven secondary amines examined were added to 5 kinds of foods and recovered from them. The mean recovery rates were in the range 71.3% (dimethylamine)-99.8% (piperidine). The limits of detection varied from 0.002 p.p.m. (dimethylamine) to 0.01 p.p.m. (morpholine).

**Harris, C. I., G. Milne, and R. McDiarmid. 1987.** The retention and metabolism of Ntau-methylhistidine by cockerels: implications for the measurement of muscle protein breakdown determined from the excretion of Ntau-methylhistidine in excreta. *British Journal of Nutrition* 57 (3): 467-478.

Excreta were collected for 4 consecutive days from cockerels 4 to 18 weeks old after subcutaneous injection of Ntau- [14CH3]methylhistidine. The recoveries of radioactivity in excreta were incomplete and progressively decreased with increasing age. Most of the radioactivity not recovered in excreta after 4 days was found in skeletal muscle where more than 55% of the radioactivity present was in the Ntau-methylhistidine- containing dipeptide, balenine. This peptide seemed to be relatively stable so that most of the labelled Ntau- methylhistidine incorporated was not released during the period of the recovery measurements. The total pool of non-protein bound Ntau-methylhistidine (free Ntau-methylhistidine + balenine) in pectoral and mixed thigh muscles increased with age and relative to the daily excretion of Ntau-methylhistidine. At 18 weeks the pool was 3.3 times the daily excretion of Ntau-methylhistidine. These observations account for the decreasing recoveries of radioactivity in excreta, due to progressive dilution of labelled Ntau-methylhistidine in an expanding pool of non- protein-bound Ntau-methylhistidine, part of which was relatively stable. It is concluded that excretion of Ntau-methylhistidine by cockerels 4 to 18 weeks old cannot be used as a reliable index of muscle protein breakdown in vivo.

**Harris, C. I., and G. Milne. 1987.** The identification of the N tau-methyl histidine-containing dipeptide, balenine, in muscle extracts from various mammals and the chicken. *Comp. Biochem. Physiol. B 86 (2): 273-279.*

**Hasegawa K, and N. Okamoto. 1978.** Lysinoalanine in the alkali-treated food proteins. *International-Congress-of-Food- Science-&-Technology-Abstracts, p.280*

Newly developed GLC and GLC-MS procedures were used in an investigation of the presence and formation of cross-linked amino acids (e.g. lysinoalanine, lanthionine) in food proteins; such acids are known to be formed by treating proteins with dil. alkaline solutions. Alkali-treated soybean globulin (cold

insoluble fraction), casein and lysozyme formed appreciable amounts of lysinoalanine, using NaOH concn. of 0.1-0.2N at 80 degree C for 16 h. Pidan (alkali-treated chicken egg) contained small amounts of lysinoalanine and lanthionine. [See FSTA (1979) 11 2A60.].

**Hayase, F., and H. Kato. 1985.** Maillard reaction products from D -glucose and butylamine. *Agricultural and Biological Chemistry* 49 (2): 467-473.

Equimolar aqueous solutions of D -glucose and n-butylamine were heated at 95 degree C for various times at pH 4, 6.5 and 11.48. The resulting brown solutions were extracted with ether. The volatile components in the ether extracts were analysed by gas chromatography and gas chromatography-MS, with a fused silica capillary column. The major components formed were identified as 3 alcohols, 4 N-butylpyrroles, N-butylacetamide, N- butylformamide, N-butylsuccinimide, 1 pyranone and 5- (hydroxymethyl)-2-furfural. In addition, 11 minor components were identified. The relative amount of each component changed markedly with pH. At pH 4.0, higher-b.p. heterocyclic compounds without C-C fission of glucose were largely formed, and at pH 11.48, lower-b.p. fission compounds were mainly formed. Both were observed in the reaction at pH 6.5.

**Hewitt D, J. E. Ford, and Porter JWG. 1979.** Nutritional quality of a spun soya-bean protein: comparison of biological and microbiological tests. *Qualitas Plantarum Plant Foods for Human Nutrition* 29 (1): 253-260.

The protein nutritional values of soy protein isolate (PI) and of a spun protein (subjected to mild alkali treatment) prepared therefrom (SP) were evaluated in feeding trials with rats and chicks; amino acid composition was determined by chemical analysis, methionine content and relative nutritional value were determined by Streptococcus zymogenes assay, and available lysine was evaluated using Tetrahymena pyriformis. Tables of results are given. Amino acid compositions of PI and SP differed little, except that SP contained 1 g lysinoalanine/kg, vs. only traces in PI. When the proteins were fed ad lib. to rats, PI gave better growth than SP. When fed at a restricted level, growth on PI and SP did not differ significantly. Chick feeding trials revealed no significant differences between growth on PI and on SP. Assay with S. zymogenes (predigestion with pepsin or papain) gave methionine values approx. 20% lower for SP than for PI; however, assay after predigestion with pronase showed no significant difference between SP and PI. Available lysine content measured by T. pyriformis assay was appreciably lower for SP than PI. [See FSTA (1980) 12 4A203.].

**Hino, T., T. Noguchi, and H. Naito. 1987.** Effect of gizzerosine on acid secretion by isolated mucosal cells of chicken proventriculus. *Poultry Science* 66 (3): 548-551.

Mucosal cells of the chicken proventriculus were isolated by a collagenase perfusion method and oxygen uptake by the isolated cells was measured as an index of the activity of gastric acid secretion. Oxygen consumption was increased by histamine; this effect was augmented by the coexistence of isobutyl-methylxanthine, an inhibitor of cyclic AMP phosphodiesterase and suppressed by imidazole, an activator of the enzyme. The action of histamine was inhibited by cimetidine, an antagonist of the histamine H2 receptor. These results indicate that the isolated cells retained the capacity to take up oxygen, responding to histamine via the H2 receptor and probably the cyclic AMP value. Gizzerosine (2-amino-9-(4-imidazolyl)-7-azanonanoic acid) also stimulated oxygen consumption by the isolated cells. The effect of gizzerosine was cancelled by cimetidine, suggesting that the mechanism by which gizzerosine acts on the mucosal cells is similar to that of histamine action. These observations are consistent with a previous presumption that gizzerosine causes gizzard erosion by increasing gastric acid secretion in chickens.

**Horikawa, H., T. Masumura, S. Hirano, E. Watanabe, and T. Ishibashi. 1992.** Effects of dietary gizzerosine on

calcium content in the femur of chicks. *Japanese Poultry Science* 29 (4): 221-227.

In experiment 1, male White Leghorn chickens, 1 day old, were fed on diets containing gizzerosine 2 mg/kg without or with cholecalciferol 50 experiment 2, chickens were fed on cholecalciferol-free basal diet for 19 days followed by diets containing cholecalciferol 50 µg/kg without or with histamine 200 or gizzerosine 2 mg/kg for 7 days. Ca content in the femur increased on the gizzerosine diet, but not on the histamine diet compared with the control diet. In experiment 3, chickens were fed on a diet containing histamine 2000 mg/kg for 14 days. The content of ash and Ca in the femur decreased. Results showed that supplementation of dietary gizzerosine with cholecalciferol increased ash and Ca contents of the femur, but there was no effect of histamine.

**Horikawa, H., T. Masumura, S. Hirano, E. Watanabe, and T. Ishibashi. 1992.** Optimum dietary level of gizzerosine for maximum calcium content in the femur of chicks. *Japanese Poultry Science* 29 (6): 361-367.

In experiment 1, chickens, 1 day old, were fed for 14 days on ad libitum diets containing gizzerosine 0, 0.5, 1, 2 or 4 mg/kg. Femur ash and calcium contents were highest in chickens given gizzerosine 1 mg/kg without decreasing body weight. In experiment 2, chickens were fed on diets containing gizzerosine 0 or 1 and cimetidine 0 or 150 mg/kg. Cimetidine is a H2-receptor antagonist of histamine and gizzerosine. Cimetidine blocked the stimulation of gastric acid secretion by histamine and by gizzerosine. When cimetidine was added to the diet with gizzerosine 1 mg/kg, the effect of gizzerosine on the content of ash and Ca in the femur was reduced. Gastric pH was decreased by gizzerosine and increased by cimetidine. Results suggest that acceleration of bone calcification by gizzerosine might be due to acceleration of gastric acid secretion since this effect was reduced by dietary cimetidine.

**Hurrell, R. F., Carpenter KJ, Sinclair WJ, Otterburn MS, and Asquith RS. 1976.** Mechanisms of heat damage in proteins. VII. The significance of lysine-containing isopeptides and of lanthionine in heated proteins. *British Journal of Nutrition* 35 (3): 383- 395.

Samples of defatted chicken white muscle (CWM), bovine plasma albumin (BPA) and other proteins were severely heated in the absence of carbohydrates to cause a large decrease in their fluorodinitrobenzene-reactive lysine concn. The heated samples were analysed for isopeptides, lanthionine, lysinoalanine and ornithoalanine concn. In heated CWM, epsilon-N-(beta-L-aspartyl)- L-lysine and epsilon-N-(gamma-L-glutamyl)-L- lysine isopeptides were detected; isopeptide concn. increased with increasing heat treatment. No lanthionine was detected. Heated BPA contained lanthionine but no isopeptides. Most other proteins studied formed both lanthionine and isopeptides. The digestibility and nutritional value of heated CWM were studied in rat feeding trials. [See FSTA (1976) 8 2A58 & 2A59 for part VI.].

**Hurst, W. J. 1990.** A review of HPLC methods for the determination of selected biogenic amines in foods. *Journal of Liquid Chromatography* 13 (1): 1-23.

This review summarizes HPLC methodologies used for the determination of selected biogenic amines in foods. It includes methods of extraction of these compounds, methods used to eliminate potential interfering compounds and the HPLC determinant step. Foods extracted include yoghurt, cheese, infant formulae, beer, cocoa, chocolate, chicken, sausage, soya sauce and fish products.

**Ijomah, P., M. N. Clifford, R. Walker, J. Wright, R. Hardy, and C. K. Murray. 1991.** The importance of endogenous histamine relative to dietary histamine in the aetiology of scombrotoxicosis. *Food Additives and Contaminants* 8 (4): 531- 542.

[Dietary histamine was investigated in order to establish whether it has any causative (additive or synergistic) involvement in the production of the more severe symptoms associated with scombrotoxicosis.] Deliberately spoiled mackerel samples and mackerel samples implicated in outbreaks of scombrotoxicosis were, under medical supervision, tested blind on normal, healthy volunteers of both sexes. These experiments identified batches of fish which could induce nausea/vomiting and/or diarrhoea when 50 g samples were consumed. It was also established that the fillets in a batch were neither of equal potency nor homogeneous with respect to histamine content. Strong evidence was obtained that dietary histamine is not a major determinant of scombrotoxicosis since potency was not positively correlated with dose, and volunteers appeared to fall into susceptible and non-susceptible subgroups. However, there is no reason to suspect allergy as being solely responsible for these differences in sensitivity. It is also possible to discount body wt. as a factor. While the data suggested that females may be more susceptible than males, this effect cannot be confirmed at the present time. Studies with susceptible volunteers predosed with either placebo or H1 antagonist (chlorpheniramine, 4 mg) demonstrated convincingly that the antihistamine can abolish vomiting and diarrhoea associated with ingestion of 50 g of scombrotoxic fish. It is therefore postulated that endogenous histamine released by mast cell degranulation has a significant role in the aetiology of scombrotoxicosis, whereas the role of dietary histamine is minor. Nature and origin of the agent responsible for mast cell degranulation is being investigated.

**Ingles, D. L., Back J.F., D. Gallimore, R. Tindale, and K. J. Shaw. 1985.** Estimation of biogenic amines in foods. *Journal of the Science of Food and Agriculture* 36 (5): 402-406.

Biogenic amines in foods have been proposed as initiators of dietary-induced migraine. Studies of levels of 2- phenylethylamine, p-tyramine and the diamines, histamine, putrescine and cadaverine in a number of Australian and other foods were made by ion exchange and HPLC methods. In the latter method the amines were chromatographed as their fluorescamine derivatives. The use of such derivatives also enabled confirmation of the identity of the amine by field desorption MS. Extraction procedures were devised to give satisfactory recoveries of amines in foods and to overcome problems associated with the binding of amines to other food constituents.

**Ishida, J., M. Yamaguchi, and M. Nakamura. 1990.** High- performance liquid chromatographic determination of beta- phenylethylamine in human plasma with fluorescence detection. *Analytical Biochemistry* 184 (1): 86-89.

A simple and highly sensitive method for estimating beta- phenylethylamine in human plasma was studied. The method employs high- performance liquid chromatography with fluorescence detection. beta-Phenylethylamine and p-methylbenzylamine (internal standard) in human plasma are isolated by cation- exchange chromatography on a Toyopak SP cartridge and then converted into the corresponding fluorescent derivatives with 3, 4-dihydro-6,7-dimethoxy-4-methyl-3-oxoquinoxaline-2-carbonyl chloride, a fluorescence derivatization reagent for amines. The derivatives are separated within 30 min on a reversed-phase column, TSK gel ODS-120T, with isocratic elution, and detected fluorometrically. The detection limit of beta-phenylethylamine is 0.3 pmol/ml in plasma.

**Itakura, C., T. Kazama, and M. Goto. 1982.** Comparative pathology of gizzard lesions in broiler chicks fed fish meal, histamine and copper Japan. *Avian Pathol. 11 (3): 487-502.*

**Ito, Y., T. Noguchi, and H. Naito. 1985.** Fluorometric determination of gizzerosine, a histamine H2-receptor agonist discovered in feedstuffs, employing high-performance liquid chromatography. *Analytical Biochemistry* 151 (1): 28-31.

This method measured 0.2-5.0 µg gizzerosine in fish meal; recovery was 95-102%. Fish meals containing

more than 20 mg/kg gizzerosine induced severe gizzard erosion in chicks.

**Ito, Y., H. Sakuta, H. Takada, and A. Tanimura. 1971.** [Nitrosamines in foods. VI. Comparison of two extraction and determination methods of secondary amines.]. *Journal of the Food Hygienic Society of Japan [Shokuhin Eiseigaku Zasshi]* 12 (5): 399-403.

A comparative investigation was made of 2 methods for extraction and determination of secondary amines: one was the authors' method [see FSTA (1973) 5 5B53] and the other was Dyer's method improved by Kawabata. Analytical results by the above 2 methods were agreeable with respect to secondary amines in 12 kinds of canned foods.

**Izquierdo-Pulido, M., Marine-Font, A. and Vidal-Carou, M.C. 1994.** Biogenic amines formation during malting and brewing. *J. Food Sci.* 59: 1104-1107.

Biogenic amines (histamine, tyramine, beta-phenylethylamine, serotonin, tryptamine, putrescine, cadaverine, agmatine, spermine, and spermidine) in raw materials, worts, and beers, and their evolution during brewing were studied using HPLC. Hops and especially malt contributed to the amine contents of wort and beer. Amines were not detected in rice, used as adjunct cereal. High amine production was found during malting. Spermine and spermidine levels decreased sharply during mashing, while levels of the other amines increased, except for putrescine which did not vary. No significant changes in level of formation of amines occurred during fermentation, except for values for tryptamine and tyramine. Amount of tyramine formed ranged from 9.70 to 27.30 mg/l.

**Jadhav, S. S., and P. R. Kulkarni. 1981.** Pressor amines in foods. *Journal of Food Science and Technology, India* 18 (4): 156-157.

Some cereals, legumes, fish, vegetables, fruits and dairy products commonly consumed in India were analysed for histamine, serotonin and tyramine. Histamine was max. in ripe tomato (11.1 mug/g), followed by cow pea (9.5 mug/g). Serotonin was max. in prawn (6.5 mug/g), raw banana (5.9 mug/g), and whole soyabean flour (5.8 mug/g). Max. content of tyramine was found in apple (3.6 mug/g), Bombay duck and whole bajra flour (each 2.6 mug/g).

**Jeevanandam, M., R. H. Ali, D. H. Young, and W. R. Schiller. 1991.** Effect of nutritional therapy on polyamine metabolism in severely traumatized patients. *Nutrition Burbank* 7 (1): 39-44.

The polyamines (PA) spermidine (SD) and spermine and their precursor putrescine (PU) play a leading role in the regulation of protein, RNA and DNA synthesis. The role of PA along with other biomarkers of injury was examined in 8 victims of multiple trauma in the early post-traumatic period when they were hypermetabolic and highly catabolic. Total parenteral nutrition (TPN) was started 48 to 60 h after trauma and continued for 6 days. The basal response to severe trauma was an increase (2- to 3-fold) in urinary PU ($P = 0.05$) and SD ($P = 0.025$) values compared with normal subjects. 6 days of TPN further enhanced the basal excretion of PU (157%) and SD (137%) peaking on the third day. There was a 20% reduction in the excretion of 3- methylhistidine on the first day of TPN, but it was still 40% above normal on the sixth day. The negative nitrogen balance was improved but not reversed. Injury stimulated ribonuclease and catecholamine values were also enhanced by nutritional therapy, peaking on the first and fourth day of TPN, respectively. Results demonstrate increased levels of PA in trauma patients that correlate well with the other known measures of protein metabolic response to injury and changes during nutritional therapy. Extracellular PA levels could be used as markers of both catabolic pathology in trauma and of its response to nutritional therapy.

**Jockel, J., and H. Eisgruber.** 1988. Histamine formation in salted anchovy products. *29. Arbeitstagung des Arbeitsgebietes Lebensmittelhygiene. Dreilandertagung vom 13. bis 16. September 1988 in Garmisch-Partenkirchen. 1988, 215-224. Giessen, Germany; Deutsche Veterinarmedizinische Gesellschaft.*

Random samples of salted anchovies in brine or oil were taken by German Government food inspectors from 1985 to 1988. From 279 products tested, 24.1% had histamine values above 100 mg/kg, and values were higher in oil than in brine fillets. Influence of storage temperature was studied with samples of spiked and unspiked fillets to examine secondary histamine formation. Storage was at 8° or 20°C for 4, 8, 12 and 16 weeks. Histamine values were stable at 8°, but at 20°C they increased, depending on initial values and were higher in fillets in oil. It was concluded that histamine values were strongly influenced during processing, but secondary histamine formation could be caused by storage at temperatures above 8°C.

**Jones, G. P., D. E. Rivett, and D. J. Tucker.** 1981. The reaction of biogenic amines with proteins. *Journal of the Science of Food and Agriculture* 32 (8): 805-812.

Alkali heat treatment of soluble and insoluble proteins (lysozyme, phosvitin, alpha-casein and keratin) in the presence of biogenic amines (phenylethylamine, histamine, putrescine and spermine) resulted in the formation of novel amino acids. Isolation of these compounds was achieved by using -1-4C- labelled amines and detecting radioactive zones on electrophoretograms of the acid-hydrolysed proteins. Confirmation of identity was achieved for 2 novel amino acids which were synthesised. The mechanism was hypothesised to be the addition of amine to dehydroalanine and evidence was provided that the latter may originate, at least in some proteins, from serine residues. The yield of the novel amino acids was increased by prolonged heating, higher temp., higher pH and increased amine concn. It was concluded that the reaction conditions employed in the present study were less severe than those encountered in many domestic and commercial food processes and that the novel amino acids could be formed in situ in many foods, especially those with a high amine content.

**Joosten, H. M. L. J.** 1987. Conditions allowing the formation of biogenic amines in cheese. III. Factors influencing the amounts formed. *Netherlands Milk and Dairy Journal* 41 (4): 329- 357.

Addition of members of the Enterobacteriaceae to milk gave a max. accumulation of 2.2 mmol/kg. Hafnia alvei LN1, a lysine decarboxylating strain, was added to cheese milk: when added at 4 x 10-5 cfu/ml, the bacteria died off much more rapidly than at 4 x 10-3 cfu/ml. In the 1st month of ripening, cadaverine was formed at the same rate in each of the cheeses infected with LN1, but after 6 months the highest concn. was found in cheese containing the smallest inoculum, probably due to nitrite accumulation. Strains with decarboxylases specifically active towards phenylalanine were not found, although tyrosine decarboxylase showed slight activity towards phenylalanine. Tryptamine was not found in any cheese. [See FSTA (1988) 20 6P110 for part II.].

**Joosten HMLJ, and A. H. Weerkamp.** 1994. Formation of biogenic amines in cheese. *Voedingsmiddelen Technologie* 27 (3): 9-11.

Biogenic amines are formed by decarboxylation of amino acids. Under certain circumstances, their presence in foods in high concentrations may lead to poisoning. Studies were carried out to determine the mechanism and conditions leading to formation of biogenic amines in cheese. Starter cultures used for cheesemaking in the Netherlands did not have the ability to produce biogenic amines. Several bacterial contaminants occasionally present in cheese or raw milk could produce biogenic amines. Heterofermentative lactobacilli, which have the ability to grow in cheese to high numbers, could also produce biogenic amines. The microbiological quality of raw milk was not an important factor in the formation of biogenic amines because pasteurization effectively inactivated the decarboxylating bacteria present.

**Kakkar, R. K., and V. K. Rai. 1993.** Plant polyamines in flowering and fruit ripening. *Phytochemistry* 33 (6): 1281-1288.

The role of polyamines in plant flowering and fruit ripening is reviewed. Aliphatic polyamines are polycationic organic compounds that have been implicated in a wide variety of biological processes. Accumulation of polyamines or hydrocinnamic acid conjugates could be species-specific and may also depend upon the conditions of the culture and parental tissues. Fruiting and endogenous polyamine contents are closely related and polyamine levels seem to be involved in fruit development. Ethylene and polyamines show opposite effects in relation to fruit ripening. A relationship between polyamines and shelf life of fruits has also been reported. [From En summ.].

**Kalninya, I. E., R. K. Biuma, and L. V. Ivanova. 1993.** Rapid method of estimating histamine in meat products. *Voprosy- Pitaniya. 1993 ( 5): 59-61.*

A rapid, highly sensitive and specific fluorimetric method of estimating histamine in meat products based on its reaction with o-phthalic aldehyde is described. Ethanol is used instead of methanol for extraction. Limits of detection are 2.6 to 2.8 ng/ml and 2 to 3 mg/kg. Recovery of histamine at concentrations of 0.025 to 0.25 µg/ml was 96.3±2.7%.

**Katsu, T., T. Kayamoto, and Y. Fujita. 1990.** Amino acid analysis using amine-sensitive membrane electrodes. *Analytica Chimica Acta* 239 (1): 23-27.

Estimation of specific amino acids (L-tyrosine and L- phenylalanine) is described, based on the detection of the amine formed by the enzymatic reaction of an amino acid with decarboxylase, using an amine-sensitive membrane electrode. The corresponding amines and phenethylamine, respectively, were selectively detected by using a poly(vinyl chloride)-based membrane electrode containing sodium tetrakis [3,5-bis (trifluoromethyl) phenyl] borate as an ion exchanger and tricresyl phosphate as a solvent mediator. The detection limits of L-tyrosine and L- phenylalanine were 20 and 20 µM respectively. The response characteristics of electrodes were compared by changing ion exchangers and solvent mediators.

**Kawamura, T., Sakai, K., Miyazawa, F., Wada, H., Ito, Y. and Tanimura, A. 1971.** [Nitrosamines in foods. V. Distribution of secondary amines in foods. 2.]. *J. Food Hyg. Soc. Japan [Shokuhin Eiseigaku Zasshi]* 12: 394-398.

A higher content of dimethylene was detected in whale meat than in mutton, chicken, pork and beef. Sausages consisting of pork, whale, mutton and tuna had a larger amount of dimethylamine than did bacon and ham. Modified powdered milk showed 5 times as much dimethylamine as did milk (approx. 0.12 vs. 0.025 mumol/g), while trace amounts of dimethylamine ( less than 0.02 mumol/g) were found in butter and processed cheese. In the case of mackerel and cuttlefish, the boiled, roasted, canned or dried samples contained more secondary amines than did the raw samples.

**Kawamura, T., Sakai, K., Miyazawa, F., Wada, H., Ito, Y. and Tanimura, A. 1971.** [Nitrosamines in foods. IV. Distribution of secondary amines in foods.]. *J. Food Hyg. Soc. Japan [Shokuhin Eiseigaku Zasshi]* 12: 192-197.

Distribution and amounts of secondary amines in foods, especially in Japanese foods, were described. Nitrosamines in foods were also analysed. Fishes and roes contained large quantities of secondary amines, i.e. dimethylamine and diethylamine, and the amounts of these amines in roasted fish increased remarkably. Only traces of secondary amines were found in meats, even in roasted meats. Nitrosamine was

not detected in any of the analysed foods.

**Keirs, R. W., and L. Bennett. 1993.** Broiler performance loss associated with biogenic amines. *Pages 31-34 in Proc. Maryland Nutr. Conf. Feed Manuf., College Park, Md.*

**Kelly, D., O'Brien, J.J. and McCracken, K.J. 1990.** Effect of creep feeding on the incidence, duration and severity of post-weaning diarrhoea in pigs. *Res. Vet. Sci.* 49: 223-228.

The effect of creep feeding on the response of pigs weaned at 2 or 3 weeks old to infection with an enteropathogenic strain of Escherichia coli (0149;K91[B]), K88 a, c [L] was studied in 2 separate experiments. Gastric intubation was adopted to regulate the intake of creep feed during the sucking period. Animals were given the enteropathogenic strain either before weaning or at weaning and the course and outcome of the infection followed bacteriologically and clinically. The response of the animals to the infection varied considerably in accord with recent reports of multiple phenotypes (to K88 + ve organisms) among pigs but consumption of creep feed before weaning did not significantly affect the prevalence, during or severity of the diarrhoea induced experimentally by the organism. Hence reputed immunological reponses mounted against dietary antigens did not predispose to or protect against this infection.

**Kelly, D., T. P. King, D. S. Brown, and M. McFadyen. 1991.** Polyamine profiles of porcine milk and of intestinal tissue of pigs during suckling. *Reproduction, Nutrition, Development* 31 (1): 73-80.

Previous studies have suggested that luminal polyamines can directly influence intestinal differentiation of neonatal rats. This investigation demonstrated the presence of high levels of polyamines in porcine milk and in the intestinal tissues of sucking piglets. The quantities of polyamines in sow milk sampled between wk 1 and 8 of lactation were determined using HPLC. The concn. of milk spermidine (SPD) remained constant over the first 3 to 4 wk of lactation but increased 4-fold between wk 4 and 7. Neither putrescine nor spermine (SPN) were detected in any of the milk samples. During intestinal development the mucosal SPD:SPN ratio was elevated between wk 1 and 3, and 5 and 7. The latter period of increase corresponded with the surge in milk SPD concn. It is suggested that milk SPD is taken up from the intestinal lumen and contributes to intestinal differentiation during the latter part of the suckling period.

**Kielwein, G., and J. Baatz. 1988.** Recent findings on the significance and formation of biogenic amines in foods. *29. Arbeitstagung des Arbeitsgebietes Lebensmittelhygiene. Dreilandertagung vom 13. bis 16. September 1988 in Garmisch- Partenkirchen. 1988, 205-214. Giessen, Germany;*

Biogenic amines formed in foods by decarboxylation of amino acids, transamination of aldehydes and ketones and degradation of nitrogenous compounds are discussed, together with microbial influences and analytical methods.

**Kirschbaum, J., B. Luckas, and W. D. Beinert. 1994.** Application of automatic pre-column derivatization with 9-fluorenylmethyl chloroformate for determination of biogenic amines and amino acids in food. *Deutsche Lebensmittel Rundschau* 90 (7): 224-228.

**Klostermeyer H, and R. J. Fritsch. 1981.** [Lysinoalanine (LAL): definition, formation, occurrence, trends.] Lysinoalanin (LAL) - Begriff, Entstehung/Vorkommen, Tendenzen. *Molkerei Zeitung Welt der Milch* 35 (36): 1137-1141.

Aspects covered in this review include: definition of LAL (chemical structure given); results of animal trials; and LAL contamination levels in milk and dairy products, and effects of processing techniques.

**Krizek, M. 1991.** The determination of biogenic amines in silage. *Archives of Animal Nutrition* 41 (1): 97-104.

A high performance liquid chromatography method for estimating biogenic putrescine, cadaverine, spermidine, spermine and histamine in silage is described, suitable for laboratories equipped with simple isocratic HPLC apparatus with photometric detectors. The amines are estimated as N-substituted benzylamides in concentrations up to 1-5 mg/kg fresh silage.

**Kuba, K., S. Miyazaki, and Y. Umemura. 1983.** Contents of free histidine and histamine in fish meals and in the model compounds and their toxicities to induce gizzard erosion. *National Institute of Animal Health Quarterly* 23 (2): 69-70.

Content of histidine and histamine was estimated in different fish meals before and after heating, and in casein or vegetable protein mixed with histidine, at intervals during heating. The poultry gizzard erosion scores of the heated fish meal and the model mixtures were noted. Whole meal of fresh sardine had a high content of histidine; whole meal of rancid sardine had a high content of histamine. Incidence of gizzard erosion was higher with meal from fresh than from rancid sardine. The content of histidine, not of histamine, was related to the degree of toxicity of the fish meal. There was non-enzymatic formation of histamine during heating of the casein or vegetable protein model mixtures.

**Lakritz, L., Spinelli, A.M. and Wasserman, A.E. 1975.** Determination of amines in fresh and processed pork. *J. Agricul. Food Chem.* 23: 344-346.

The concn. of a number of amines was determined in fresh, cooked, smoke-cured, and putrefied pork. Analyses were conducted for spermine, spermidine, putrescine, cadaverine, histamine, tyramine, tryptamine, and ethanolamine. The amines were recovered from perchloric acid extracts of lean meat and derivatized with 1-dimethylaminonaphthalene-5-sulphochloride. The fluorescent derivatives were separated by TLC, extracted, and then quantiated spectrofluorometrically. The concn./100 g of fresh tissue ranged from 0.5 mg for tyramine to 189 mg for putrescine. Significant increases in spermine, sperkidine, putrescine, and cadaverine occur during putrefaction. Cooking at 71 degree C decreases the concn. of amines.

**Lancaster, F. E., and J. F. Lawrence. 1989.** Determination of total non-sulphonated aromatic amines in the food colour amaranth by dithionite reduction followed by derivatization and high- performance liquid chromatography. *Food Additives and Contaminants* 6 (4): 415-423.

A method is reported for detn. of total free and bound non- sulphonated aromatic amines (NSAA) in the food colour amaranth. Bound amines are first reduced using sodium dithionite, then all NSAA are extracted into chloroform, followed by transfer to aqueous acid solution, diazotization with sodium nitrite and subsequent coupling with 2-naphthol-3,6-disulphonic acid, disodium salt (R-salt). The coloured derivatives are then analysed by reversed-phase ion-pair liquid chromatography using an absorbance detector at 522 nm. Samples of amaranth were spiked with various amounts of 1- and 2-naphthylamine and recoveries ranged from 80 to 100% with deviations of less than 2%. A survey of commercial amaranth samples indicated that the dye may contain up to 435 mug/g of total 1-naphthylamine and 214 mug/g of total 2-naphthylamine. The majority of NSAA are bound to R-salt during the manufacturing process and less

than 5% remain in the free state in the dye.

**Langhans, W., R. Harlacher, G. Balkowski, and E. Scharrer. 1990.** Comparison of the effects of bacterial lipopolysaccharide and muramyl dipeptide on food intake. *Physiology and Behavior* 47 (5): 805-813.

For further characterization of the mechanism involved in anorexia during bacterial infection, the question as to whether muramyl dipeptide (MDP), the minimal immunologically active structure of Gram-positive bacterial cell walls, affects rats' food intake in the same way as lipopolysaccharide (LPS) from Escherichia coli was studied. MDP (1.6 mg/kg body weight) injected intraperitoneally (IP) reduced food intake by decreasing meal frequency without affecting meal size. Indomethacin (2.5 mg/kg IP) but not verapamil (5 mg/kg) attenuated the hypophagic effect of MDP. In further experiments, MDP and LPS (100 µg/kg IP) both inhibited gastric emptying and indomethacin did not block this effect of LPS. Hepatic vagotomy did not attenuate the hypophagic effects of MDP or LPS. LPS reduced water intake only when food was available, but reduced food intake also during water deprivation. MDP did not affect water intake. MDP and LPS both had an aversive effect, but LiCl, which was also aversive, did not reduce feeding in the conditions tested. This questions the role of a conditioned taste aversion in the hypophagia and may therefore also contribute to anorexia during infection. In contrast, an inhibition of gastric emptying, an activation of hepatic satiety signals or a reduction of water intake, does not seem to be crucial for the hypophagic effects of MDP or LPS.

**Lawrence, R. A., and Jelen P. 1983.** Alkaline extraction of protein from residues of mechanical separation of poultry. *Pages 50-51 ,vol.2 Proc. 6th International Congress of Food Science and Technology.*

A slurry of mechanically separated poultry bone residues was extracted at pH 9.5-11.5 for 30 min at 22 degree C, followed by centrifugation. Protein was precipitated from the supernatant by pH adjustment to 4.5-6.5 with various acids, and separated by centrifugation. Total protein extracted did not increase with pH greater than 10.5, and fell sharply below pH 9.5. Differences between acid types in TS of precipitate were minimal. Lysinoalanine was not formed under optimum extraction conditions, at pH 10.5. [See FSTA (1984) 16 9A640.].

**Lawrence, R. A., and Jelen P. 1982.** Formation of lysino-alanine in alkaline extracts of chicken protein. *Journal of Food Protection* 45 (10): 923-924.

Bone residues from mechanical deboning of chicken backs, necks and spent layers were extracted at pH 9.2, 10.0, 10.7 and 11.5. The centrifuged liquid protein extracts were kept at 22 degree , 35 degree and 50 degree C for 1, 4 and 16 h. Detn. of lysino- alanine (LAL) were made after freeze-drying and fat extraction of the treated samples. No LAL was detected in any samples treated for 1 h. Samples treated for 4 h showed measurable amounts of LAL only at pH 11.5 at all 3 temp. used, and at pH 10.7 at 50 degree C. After 16 h, LAL was produced at all pH treatments at 50 degree C; small amounts were also formed at 22 degree and 35 degree C at pH 10.7 and 11.5. It is concluded that the proposed alkali extraction procedure would not produce LAL in the protein extract under technologically optimal conditions.

**Liener, I. E. 1994.** Implications of antinutritional components in soybean foods. *Crit. Rev. Food Sci. Nutr. 34 (1): 31-67.*

There are a number of components present in soybeans that exert a negative impact on the nutritional quality of the protein. Among those factors that are destroyed by heat treatment are the protease inhibitors and lectins. Protease inhibitors exert their antinutritional effect by causing pancreatic hypertrophy/ hyperplasia, which ultimately results in an inhibition of growth. The lectin, by virtue of its ability to bind to

glycoprotein receptors on the epithelial cells lining the intestinal mucosa, inhibits growth by interfering with the absorption of nutrients. Of lesser significance are the antinutritional effects produced by relatively heat stable factors, such as goitrogens, tannins, phytoestrogens, flatus-producing oligosaccharides, phytate, and saponins. Other diverse but ill-defined factors appear to increase the requirements for vitamins A, B12, D, and E. The processing of soybeans under severe alkaline conditions leads to the formation of lysinoalanine, which has been shown to damage the kidneys of rats. This is not generally true, however, for edible soy protein that has been produced under milder alkaline conditions. Also meriting consideration is the allergenic response that may sometimes occur in humans, as well as calves and piglets, on dietary exposure to soybeans.

**Lin, J. K., Y. J. Lee, and H. W. Chang. 1983.** High concentrations of dimethylamine and methylamine in squid and octopus and their implications in tumour aetiology. *Food and Chemical Toxicology* 21 (2): 143-149.

Naturally occurring amines (ammonia, methylamine, ethylamine and dimethylamine) were determined by HPLC, in squid, octopus and 17 other seafoods purchased from supermarkets and fisheries in Taipei. Ammonia and dimethylamine were found in all of the seafoods tested, and particularly high levels of dimethylamine (946-2043 p.p.m.) and methylamine (38-255 p.p.m.) were detected in various spp. of squid and octopus. An aqueous extract of squid reacted with nitrite in acidic medium to yield N-nitrosodimethylamine. Dimethylamine in dried squid tissue was readily extracted with water or 1% sodium carbonate solution. Heat treatment of dried squid at 200 degree C was found to increase its amine content dramatically, possibly due to pyrolytic decarboxylation of some amino acids. These findings add to the evidence that dietary factors may have an important role in the aetiology of stomach cancer and other gastro-intestinal tumours in Japan, since the traditional Japanese diet is rich in dimethylamine and in nitrate.

**Lovenberg, W. 1974.** Psycho- and vasoactive compounds in food substances. *Journal of Agricultural and Food Chemistry* 22 (1): 23-26.

A number of biogenic amines that participate in mammalian physiology are also found to occur in food substances. The origin and potential toxicity of these compounds are reviewed. The presence of psychoactive substances in food and plant products is also examined.

**Luk, G. D. and Baylin, S. B. 1983.** Poly amines and intestinal growth increased poly amine biosynthesis after jejunectomy. *Am. J. Physiol.* 245: G656-???.

Transient increases in the activities of ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (SAM-DC), key enzymes in polyamine biosynthesis, may be critical to initiation of cell growth. Such increases in ODC ( 170X) and SAM-DC ( 83X) activities, and their synthetic products putrescine ( 4X) and spermidine ( 2X), occur in rat ileal mucosa between days 1-4 after 50% intestinal reaction. This is the time period of initiation of mucosal cell hyperplasia in intestinal adaptation after resection and is characterized by increased mucosal cell proliferation, as measured morphologically and biochmically. Intestinal weight increased by 76% and mucosal thickness by 48%. Mucosal DNA content increased by 67% and mucosal DNA synthesis by 104%. Increased intestinal crypt cell proliferation was manifested by a 120% increase in labeling per crypt and a 152% increase in crypt cell production rate (CCPR). The increase in ODC activity was closely associated with the increases in CCPR and rate of villus lengthening. Rates of mucosal cell proliferation, as measured by CCPR, and villus and crypt lengthening were significantly correlated with ODC activity ($r = 0.97$, 0.98 and 0.94, respectively; $P < 0.01$ for all). The increase in ODC activity, SAM-DC activity and polyamine biosynthesis is apparently closely associated with the process of adaptive postresectional crypt cell proliferation.

**Machin, D. H., S. Panigrahi, J. Bainton, and T. R. Morris. 1990.** Performance of broiler chicks fed on low and high oil fish silages in relation to changes taking place in lipid and protein components. *Animal Feed Science and Technology* 28 (3-4): 199-223.

Changes in the protein and lipid components of fish silages made from low-oil content fish (LOF; whiting, Gadus merlinger) and high-oil content fish (HOF; mackerel, Scromber scombus) were studied during ensiling, drying and subsequent storage. Chicken.

**Maga, J. A. 1978.** Amines in foods. *CRC Crit. Rev. Food Sci. Nutr.* 10 (4): 373-403.

Amines represent a class of compounds which historically have been associated with fishery products and cheese; however, this review clearly demonstrates that their occurence is widespread in foods. In addition, their formation pathways, methods of isolation and identification, and sensory properties are discussed.

**Maga, J. A. 1978.** Lysinoalanine in foods. *Journal of Agricultural and Food Chemistry* 32 (5): 955-964.

The chemistry of lysinoalanine (LAL) formation is reviewed, as are also occurrence in foods (LAL contents in many common foods and ingredients are tabulated), inhibition of LAL formation, methods of analysis, and biological effects. Areas requiring future research are indicated.

**Makarios-Laham, I.K. and Lee, T.C. 1993.** Protein hydrolysis and quality deterioration of refrigerated and frozen seafood due to obligately psychrophilic bacteria. *J. Food Sci.* 58: 310-313.

[Protein hydrolysis and quality deterioration of refrigerated and frozen sea food, due to obligately psychrophilic bacteria were studied.] 2 obligately psychrophilic marine Vibrio spp. (MV-3 and MV-6) hydrolysed proteins and caused deterioration of refrigerated and frozen sea foods (fresh and cooked shrimp, haddock fillets and scallops). Protein hydrolysis was determined after storage at 4 degree C and -20 degree C and reported as % increase over uninoculated controls stored under the same conditions. When fresh shrimp was inoculated with isolate MV-3, increases in protein hydrolysis were 19.2% after 2 wk refrigeration and 14.2% after 12 wk frozen storage. Thus, isolates were capable of hydrolysing protein and causing a deterioration in the quality of refrigerated or frozen fish and shellfish.

**Mayer, K., Pause, G. and Vetsch, U. 1974.** [Fermentation development and histamine production during sauerkraut manufacture.] Gaerverlauf und Histaminbildung bei der Sauerkrauterzeugung. *Mitteilungen Aus Dem Gebiete Der Lebensmitteluntersuchung Und Hygiene* 65: 234-238.

Attempts were made to determine pH-values, total acid and histamine contents as well as changes in the bacterial flora in two containers of fermenting sauerkraut. Simultaneously with the appearance of lactic acid cocci (Pediococcus cerevisiae) the histamine contents increased; after 10 wk histamine levels of 160 mg/kg were found. The addition of 1 ppm manganese had no influence on the fermentation velocity.

**Masumura, T., M. Sugahara, T. Noguchi, K. Mori, and H. Naito. 1985.** The effect of gizzerosine, a recently discovered compound in overheated fish meal, on the gastric acid secretion in chicken. *Poultry Science* 64 (2): 356-361.

The effect of gizzerosine (2-amino-9-(4-imidazolyl)-7- azanonanoic acid), which causes gizzard erosion in

chicks, on gastric acid secretion was studied in chicks 3 days old. The broiler chicks given a diet containing synthetic DL-gizzerosine showed severe signs of gizzard erosion after 7 days. The pH of the gastric and duodenal contents of those chicks was lower than that of the control chicks, showing that acid secretion was increased in gizzerosine-fed chicks. The pH of gastric contents was lower and the amount of total gastric acid was higher in chicks starved for 1 day and given gizzerosine by vein than in those given physiological saline solution. Cimetidine, H2- receptor antagonist of histamine, given by muscle blocked the stimulation of acid secretion by gizzerosine and by histamine. Cimetidine added in feed also prevented gizzard erosion, which was induced by feeding on overheated mackerel meal. The results showed that gizzard erosion developed after an oral or intravenous load of gizzerosine is caused, at least in part, by the increased gastric acid secretion.

**Matsuzaki, S., K. Hamana, and K. Isobe. 1990.** Occurrence of N6-methylagmatine in seeds of leguminous plants. *Phytochemistry* 29 (4): 1313-1315.

When analysing polyamines in seeds of various leguminous plants by HPLC, several unknown peaks were observed. One of the unknowns was identified as N6-methylagmatine from its behaviour on HPLC and TLC and its susceptibility to agmatine oxidase and alkaline hydrolysis. N6- Methylagmatine was present in seeds and seedlings of soyabeans, Psophocarpus tetragonolobus, peas, Medicago sativa, Phaseolus vulgaris and groundnuts among 20 species of leguminous plants tested.

**Mayer, K. April 1976.** [Biogenic amines in foods. Studies on wine and sauerkraut.] Biogene Amine in Lebensmitteln. Eigene Untersuchungen in Wein und Sauerkraut. *Qualitas Plantarum Plant Foods for Human Nutrition* 26 (1/3): 263-269.

A brief account is given of studies on formation of histamine and other toxic biogenic amines in wines. Analysis of 450 wine samples showed the mean histamine content of red wines to be 3.3-3.8 mg/l., vs. 1.1-1.2 mg/l. for white wines. 26% of red wines and 5% of white wines had histamine concn. greater than 5 mg/l. Studies on a further 282 wines showed that histamine concn. increased with increasing wine pH; histamine concn. was also closely related to the count of Pediococcus cerevisiae. Significant concn. of tyramine, phenylethylamine, putrescine and cadaverine were also detected in wines. A brief account is also given of formation of histamine during sauerkraut fermentation. Pediococcus cerevisiae commonly appears if the sauerkraut reaches pH less than 4.0, and may produce large quantities of histamine (less than200 mg/kg) and other volatile amines. To avoid this, pasteurization of the sauerkraut when it reaches pH 4.0 is recommended. [See FSTA (1977) 9 10J1369.].

**McCorkle, F. M., and R. L. Jr. Taylor. 1993.** Biogenic amines regulate avian immunity. *Poultry Sci. 72 (7): 1285-1288.*

Alzet mini-osmotic pumps were implanted subcutaneously to administer norepinephrine (NE) and epinephrine (E) to 6-wk-old line UNH 105 chickens. Dose-time studies showed the most effective NE and E dose and exposure time on two chicken cellular immune responses: the phytohemagglutinin (PHA) wattle response and leukocyte migration. Administration of 1 micrograms/h NE for 72 h suppressed significantly the wattle stimulation index [2.48 +/- .3 (SE)] compared to that of saline controls (4.1 +/- .3) but enhanced mean leukocyte migration (7.7 +/-.3 versus 4.9 +/-.3). Epinephrine at 1 micrograms/h for 72 h significantly suppressed the wattle index (1.8 +/-.2) compared to that of controls (2.8 +/ -.3) but E at 1 micrograms/h for 48 h enhanced leukocyte migration (9.5 +/- .2 versus 6.4 +/- .2). Continuous administration of NE and E at physiological levels alters cell- mediated immunity and appears to have an immune regulatory role in the chicken.

**Mercer, L. P., S. J. Dodds, M. D. Weber, and J. D. Dunn. 1990.** Histidine, histamine, and the neuroregulation of food intake: a review and hypothesis. *Nutrition Burbank* 6 (4): 273- 277.

Modulation of feeding behaviour is a normal part of survival, but certain pathological conditions interrupt or modify regulatory aspects of feeding, leading to inappropriate intake. This review examines aspects of metabolism, particularly that of histidine, associated with the anorexia seen in animals suffering from protein-energy malnutrition (PEM). In kwashiorkor- like PEM, histidine concentration is increased in blood plasma and the brain, whereas concentrations of other essential amino acids are decreased. The PEM-induced increase in brain histamine is 5-fold. This increase may affect the central nervous system via histamine, whose synthesis rate is related to histidine concentration. In children, PEM consistently depresses food intake and causes oedema, growth failure and psychomotor changes. Histamine stimulates ACTH and corticosteroid release. Based on these observations, it is hypothesised that one component of the pathophysiological neuroregulation of food intake involves histidine-induced variation of histamine concentration in the hypothalamus and the subsequently altered neurochemical activity at the corticotropin-releasing factor neurons of the paraventricular nucleus.

**Miller, A. III, Scanlan, R.A., Libbey, L.M., Petropakis, H. and Anglemier, A.F. 1973.** Quantitative determination of dimethyl- and trimethylamine in fish protein concentrate. *J. Agr. Food Chem.* 21: 451-453.

Dimethylamine (DMA), 25-150 ppm, and trimethylamine (TMA), 5-10 ppm, were detected in samples of fish protein concentrate (FPC) prepared from frozen red hake (Urophycis chuss) and Pacific hake (Merluccius productus) by isopropyl alcohol extraction. The following compounds were also identified by combined GLC-MS: methyl mercaptan, acetaldehyde, propionaldehyde, methylene chloride, acetone, chloroform, isopropyl alcohol, ethyl alcohol, butanone, toluene, dimethyl sulphide, and dimethyl disulphide. The probable presence of ethylamine and a butylamine was indicated.

**Miyazaki, S., and Y. Umemura. 1987.** Effects of histamine antagonists, an anticholinergic agent and antacid on gizzard erosions in broiler chicks. *British Poultry Science* 28 (1): 39- 45.

Diphenhydramine, an H1-antagonist, had no effect on gizzard erosion (GE) induced by heated casein-histidine mixture (h-CH) but reduced the severity of the lesions induced by histamine and starvation. Cimetidine, an H2-antagonist, blocked completely the formation of the lesions induced by h-CH or histamine but did not prevent starvation-induced GE. Gastric antacid decreased the severity of GE caused by h-CH and histamine. The formation of GE by starvation was blocked by the administration of propantheline bromide or inert solids. The results suggest that the stimulated gastric secretion caused by the H-2-activity of h-CH or histamine is largely responsible for the formation of GE. In starvation- induced GE, however, alteration of gastric secretion had no effect on the formation of the lesion as it was caused by the emptiness of the gizzard.

**Moret, S., R. Bortolomeazzi, M. Feruglio, and G. Lercker. 1992.** Determination of biogenic amines in Italian cheeses. *Scienza e Tecnica Lattiero Casearia* 43 (3): 187-198.

Biogenic amine compositions were determined in 16 Italian cheeses (2 replicates for a total of 32 samples). The most abundant amines were tyramine (tyr) and histamine (hist). Gorgonzola cheese had the highest concn. of tyr (146.0 mg/100 g), followed by Asiago Stravecchio (29.1 mg/100 g) and by 2 samples of Montasio cheese (23.3 and 17.7 mg/100 g resp.). Hist was highest in Gorgonzola (84.6 mg/100 g), Asiago Stravecchio (79.0 mg/100 g), Parmigiano Reggiano (12.4 mg/100 g) and Sweet Provolone (8.2 mg/100 g). In order of concn., the other amines detected were cadaverine, putrescine, tryptamine and 2-phenylethylamine. For some type of cheeses, these amines were present in considerable amounts.

**Murase M, and F. Goto. 1977.** [Formation of lysinoalanine resiues during alkaline treatment of egg white.]. *Journal of Japanese Society of Food Science and Technology [Nippon Shokuhin Kogyo Gakkaishi]* 24 (11): 547-552.

An amino acid analyser was used to determine lysinoalanine (LA) in commercial pidans (alkali-treated eggs) and in laboratory- prepared chicken and quail pidans. LA levels found were 2.8-9.3 mM /100 g protein in egg white and 2.9-8.7 mM /100 g protein in egg yolk. Studies of LA formation in chicken egg white during heating in water for less than30 min indicated that LA formation increased with increasing time, temp. and pH. Na2CO3 was found to play a specific role in LA formation. [From En summ.].

**Murray, C. K., and D. M. Gibson. 1972.** An investigation of the method of determining trimethylamine in fish muscle extracts by the formation of its picrate salt. *Journal of Food Technology* 7 (1): 47-51.

TLC, GLC and colorimetric procedures were used to identify and determine amines present in standard solutions, cod muscle extracts and in the toluene phase of a modified trimethylamine (TMA) procedur [see preceding abstr.]. KOH (45%) or $K_2CO_3$ (50%) was used to liberate the base from the formaldehyde treated 5% trichloroacetic acid extracts. When KOH was used as alkali, TMA only was detected; when $K_2CO_3$ was used, dimethylamine (DMA) was detected in addition to TMA. Although this test was qualitative, the results indicated that some DMA was not fixed by formaldehyde in the presence of $K_2CO_3$ and was transferred to the toluene layer and thereby gave rise to apparently high TMA values. Thus, differences in TMA-N values found for cod with KOH and $K_2CO_3$ in the picric acid procedure were due to interference by DMA in the analysis. The difference was small in iced fish but could be large in frozen fish where greater quantities of DMA could be formed. DMA may be a useful index of the quality of frozen fish. It is concluded that in applications of the picric acid method for estimating TMA in fish tissues, 45% KOH gave better recoveries and reproducibility than 50% $K_2CO_3$ and there was little interference by DMA. It is noted that further work is necessary to determine the extent of DMA interference in the TMA-N values of other species of fish.

**Nagayama, T., Y. Tamura, T. Maki, K. Kan, Y. Naoi, and T. Nishima. 1985.** [Non-volatile amines formation and decomposition in abusively stored fishes and shellfishes.]. *Journal of Hygienic Chemistry [Eisei Kagaku]* 31 (6): 362-370.

The formation of histamine (His), cadaverine (Cad), putrescine (Put), spermidine (Spd), tyramine (Tyr) and agmatine (Agm) during the storage of 6 types of seafood (mackerel, tuna, sardine, Pacific saury, shortneck clam, and boiled octopus) at 27 degree C was investigated using HPLC. Changes in pH and volatile base nitrogen (VBN) were also measured. VBN increased more sharply as putrefaction progressed in tuna, mackerel and octopus than in the other 3 seafoods. The pH rose very gradually from approx. 6.0 to 7.5 in all the seafoods examined except octopus when it fell to 4.0. Agmatine was not detected in any of the seafoods, and changes in the amines in Pacific Saury were very small. Histamine levels rose steeply for the 1st 2 days of storage in tuna and mackerel but declined on the 3rd day. A similar effect was observed with putrescine in octopus. Results generally showed that amine levels were a good indicator of food quality because they started to form before any visible signs of decomposition occurred and prior to changes in pH and VBN levels. [From En summ. and tables].

**Nagendra, T. A., Indrani Karunasagar, and I. Karunasagar. 1990.** Levels of histamine in some of the commercially important fish and fishery products in India. *Pages 112-120 in FAO Fisheries Report No. 401, suppl., 7th Session of the Indo-Pacific Fishery Commission Working Party on Fish Technology and Marketing.*

Histamine was estimated in a number of raw and processed fish samples. Among raw fish, histamine concentrations ranged between 26.55 to 59.85 mg% for mackerel, 8.31 to 41.56% for sardines, 4.5 to 23.2 mg% for seerfish, 2.8 to 13.5 mg% for lesser sardines. Among processed fish, salted and dried mackerels had high histamine concentrations (13.3 to 58.5%) and a sample of canned tuna in oil had 66.5 mg% but canned sardines and mackerels had less than 20 mg%. Histamine in raw fish was not correlated directly

with the count of histamine decarboxylating bacteria suggesting that all of them are capable of forming histamine in fish. A study of the microbiological profile of mackerels at various stages of salting and drying did not indicate that histamine-producing organisms would proliferate in fish during the initial stages of drying at ambient temperature.

**Nakamura, M., Wada, Y., Sawaya, H. and Kawabata, T. 1979.** Polyamine content in fresh and processed pork. *J. Food Sci.* 44: 515-517.

A survey on the occurrence of 4 polyamines, spermine, spermidine, putrescine and cadaverine, in fresh and processed pork was conducted. The effect of deterioration on the concn. of these polyamines in pork was examined, and, in addition, detn. was made of these 4 polyamines in various portions of swine rejected on the basis of having tumours. The concn. of polyamines/100 g fresh and processed pork purchased in the areas of Tokyo and Kanagawa Prefecture ranged from 2.0 to 6.9 mg for spermine, 0.1 to 0.9 mg for spermidine, not detected (N.D.) to 0.1 mg for putrescine, and N.D. to 0.8 mg for cadaverine. When fresh pork samples were stored at 4 degree or 20 degree C, no significant increase in polyamine content was observed until the samples reached the initial stage of decomposition. Almost no appreciable difference in the polyamine levels could be observed between the muscle of swine having embryonal nephroma or malignant melanoma, and those taken from healthy swine.

**Nakazato, M., K. Saito, S. Morozumi, T. Wauke, F. Ishikawa, K. Fujinuma, T. Moriyasu, T. Nishima, and Y. Tamura. 1994.** Determination of putrefactive non-volatile amines in foods following sample cleanup by solid phase extraction. *Japanese Journal of Toxicology and Environmental Health* 40 (2): 203-209.

An analytical method for the detection of putrescine, cadaverine, histamine, tyramine, and spermidine in foods by HPLC is described. The 5 amines were extracted from fish (horse mackerel), fish products (kusaya), miso, soy sauce, wine, and sake with 1% trichloroacetic acid. 0.1M sodium octanesulphonate was added to the extract; this solution was then passed through a Sep-Pak Vac C18 cartridge column. After washing the column with water, the amines were eluted with a methanol and water mixture (6:4). Amines in the eluate were converted into the corresponding dansyl amines by reaction with dansyl chloride under alkaline conditions. Dansyl amines were separated on a Cosmosil 5C18-AR column with a mobile phase of acetonitrile-water (65:35) and detected with a fluorescence detector (excitation 325 nm, emission 525 nm). Recoveries of the amines were greater than 79.4%. Detection limits for the amines in the samples were: 1 mug/g for putrescine, cadeverine, and spermidine; 5 mug/g for tyramine; and 20 mug/g for histamine. [From En summ.].

**Nashef, A. S., Osuga DT, Lee HS, Ahmed AI, Whitaker JR, and Feeney RE. 1977.** Effects of alkali on proteins. Disulfides and their products. *Journal of Agricultural and Food Chemistry* 25 (2): 245-251.

Alkali treatment of disulphide-containing proteins with different structures and properties resulted in the formation of similar type products, but with different energies of activation. The principal protein studied was lysozyme, with comparative studies on bovine pancreatic ribonuclease, bovine alpha-lactalbumin, bovine serum albumin, chicken ovotransferrin, and several avian ovomucoids. Alkali treatment of proteins (10--- 5M protein in 0.1M NaOH at 50 degree C for 24 h) resulted in the loss of cystine and lysine and the formation of new amino acids. Alkali treatment was accompanied by an increase in absorbance at 241 nm with time until it reached a max. at which time it started decreasing and finally plateaued. The rate of increase in absorbance at 241 nm was found to be a function of both base and disulphide concn. The mechanism of action appeared to involve a beta-elimination of the disulphides resulting in the intermediate, dehydroalanine. Michael-type nucleophilic additions of the epsilon-amino groups of lysine, the S of cysteine, and the N of ammonia to the double bond of the dehydroalanine lead to the formation of lysinoalanine, lanthionine and beta- aminoalanine, respectively. The energy of activation for several disulphide-containing proteins was in the range 14.2 kcal/mol for Golden- Amherst pheasant cross ovomucoid to 23.8 kcal/mol

for lysozyme, while the change in free energy was essentially the same (20.2 plus/minus 0.2 kcal/mol) for all proteins.

**Nasran, S., I. Setyaningsih, A. M. Anggawati, and S. Putro. 1985.** Histamine formation in boiled-salted (pindang) mackerel. *FAO Fisheries Report* 317 (Suppl.): 368-372.

Histamine formation was studied in boiled-salted (pindang) mackerel during chilled and ambient temp. storage, and the quality of pindang prepared from raw material subjected to delayed icing was assessed. Tabulated data show the results of chemical and microbiological analysis of mackerel stored for 9 days at room or chill temp., and of the boiled-salted product made from raw material stored up to 4 days at room temp. Results illustrate the significant effect of chilling on reducing histamine formation in both fresh and processed mackerel. [See FSTA (1987) 19 3R10.].

**Neurath, G.B., Duenger, M., Pein, F.G., Ambrosius, D. and Schreiber, O. 1977.** Primary and secondary amines in the human environment. *Food Cosmetics Toxicol.* 15: 275-282.

Altogether 40 primary and secondary amines with different gas-chromatographic properties have been detected in samples of fresh vegetables, preserves, mixed pickles, fish and fish products, bread, cheese, stimulants, animal feedstuffs and surface waters, and 21 of these have been identified by MS. Secondary amines, the precursors for the carcinogenic N-nitrosamines were generally found in concn. less than 10 p.p.m., although higher concn. occurred in herring preparations, some cheese and samples of large radish and red radish. Besides dimethylamine and diethylamine, the most prevalent secondary amines were found to be pyrrolidine, piperidine, N-methylbenzylamine, N-methylaniline and N-methylphenethylamine, the latter apparently being the most widespread in foods of plant origin. The highest content of secondary amines found so far was in red radishes (38 p.p.m. pyrrolidine, 20 p.p.m. pyrroline, 5.4 p.p.m. N-methylphenethylamine and 1.1 p.p.m. dimethylamine). Concn. of secondary amines found in surface waters have generally been below 15 parts/billion (15 mug/kg).

**Nout, M. J. R., M. M. W. Ruikes, H. M. Bouwmeester, and P. R. Beljaars. 1993.** Effect of processing conditions on the formation of biogenic amines and ethyl carbamate in soybean tempe. *Journal of Food Safety 13 (4): 293-303.*

Effects of manufacturing conditions, i.e. soaking, boiling, fermentation and home cooking by stewing or frying, and pure cultures of microorganisms commonly occurring in tempeh on production of toxicants such as biogenic amines and ethyl carbamate were investigated. Biogenic amines levels in soaked soybeans were low (total less than 280 p.p.m.), and not significantly affected by boiling, but increased by fermentation. Rhizopus oligosporus mainly produced tyramine and some putrescine (total biogenic amines approx. 1800 p.p.m.). With added inoculation of Klebsiella pneumoniae and Trichosporon beigelii, the total amount of biogenic amines increased slightly (2000 and 2100 p.p.m., respectively) with a shift towards cadaverine. With added Lactobacillus plantarum, a reduction of tyramine levels resulted in a considerably lower total level of biogenic amines (approx. 1000 p.p.m.). Storage at 5 degree C did not affect the level of biogenic amines, whereas at 25 degree C, increased levels of putrescine were observed. Home cooking by stewing had little effect, but frying in oil resulted in significant decreases in both putrescine and tyramine. Preventive measures to keep biogenic amines at low levels in tempeh are recommended and they include inoculation with selected lactic acid bacteria which cannot produce but can degrade biogenic amines, and frying instead of stewing of tempeh. Ethyl carbamate levels were negligible ( less than 11 p.p.b.) in all treatments; this was attributed to the absence of significant concn. of ethanol in the product.

**Nugon Baudon, L., O. Szylit, M. Chaigneau, N. Dierick, and P. Raibaud. 1985.** Production of amines in vitro and

in vivo by a strain of Lactobacillus from the crop of fowls. [Production d'amines in vitro et in vivo par une souche de lactobacille isolee d'un jabot de coq]. *Annales de l'Institut Pasteur/Microbiology* 136B (1): 63-73.

Strain LEM-207 (which closely resembled Lactobacillus acidophilus) was capable of producing tyramine in germ-free chicks. This may be toxic for the birds.

**O'Donovan, C. J. 1976.** Recent studies of lysinoalanine in alkali-treated proteins. *Food and Cosmetics Toxicology* 14 (5): 483-489.

The topic is reviewed under the following headings: lysinoalanine (LAL), a recently recognized amino acid, in alkali- treated proteins; studies of alkali-treated spun soya protein in rats; studies of Alpha protein in rats; observations with free synthetic LAL; comparative effects in rats of synthetic LAL and alkali-treated soya protein, before and after complete acid hydrolysis; studies of oligopeptide-bound LAL; absence of LAL-induced renal cytomegaly in species other than the rat; historical note on alkali treatment of foods; and ubiquity of LAL in alkali- and/or heat-treated proteins.

**Okazaki, T., T. Noguchi, K. Igarashi, Y. Sakagami, H. Seto, K. Mori, H. Naito, T. Masumura, and M. Sugahara. 1983.** Gizzerosine, a new toxic substance in fish meal, causes severe gizzard erosion in chicks. *Agricultural and Biological Chemistry* 47 (12): 2949-2952.

The histamine derivative, 2-amino-9-(4-imidazolyl)-7-azanonanoic acid, was isolated from mackerel and named gizzerosine. It caused severe gizzard erosion in chicks within a week when fed to them at 2.2 mg/kg of the diet. Gizzerosine is produced during fish meal manufacture by the reaction between histidine and fish protein.

**Patterson, R. L. S., and D. S. Mottram. 1974.** The occurrence of volatile amines in uncured and cured pork meat and their possible role in nitrosamine formation in bacon. *Journal of the Science of Food and Agriculture* 25 (11): 1419-1425.

The concn. of volatile amines were determined in the eye muscle of 10 pork carcasses immediately after slaughter, before curing, after maturation and after vacuum-packed storage of the bacon. Methylamine (MA), dimethylamine (DMA), trimethylamine (TMA), ethylamine (EA), diethylamine (DEA), n-propylamine (n-PA) and isopropylamine (iso-PA) were detected by gas chromatography; MA was present in greatest concn. (less than1900 mug/kg), the concn. of the others being considerably less. During bacon manufacture the concn. of DMA, TMA, n-PA and iso-PA increased consistently up to the end of the maturation period, despite the presence of nitrite both in brine and in bacon; in subsequent storage only DMA and TMA continued to increase. MA decreased during curing and maturation, and then remained unchanged; EA and DEA were unchanged throughout at very low levels. The mean value for DMA concn. was less than 200 mug/kg before curing; higher values (max. 520 mug/kg) were found in vacuum packed stored bacon. No nitrosamines were found above the detection limit (1 mug/kg) in any of the uncooked pork or bacon.

**Pechanek, U., H. Woidich, W. Pfannhauser, and G. Blaicher. 1980.** [Studies on occurrence of biogenic amines in foods.] Untersuchung ueber das Vorkommen von biogenen Aminen in Lebensmitteln. *Ernaehrung* 4 (2): 58-61.

Toxicity of biogenic amines occurring in foods is briefly discussed, with special reference to histamine, tyramine and phenylethylamine. Samples of cheese (Emmental, Edam, Cheddar and Hungarian ewes-milk cheese), red wine (Austrian, French and Hungarian) and fish (frozen cod, coley and hake, fresh redfish and plaice) were analysed for biogenic amines by a method involving extraction with trichloroacetic acid,

separation by ion exchange chromatography on a cation exchanger resin, reaction of the eluate with ninhydrin and spectrophotometric detn. at 570 nm. Tables of results are given. Max. values recorded in cheese were (mg/kg): putrescine 40.65 (Cheddar), histamine 110.0 (Emmental), cadaverine 42.45 (Cheddar) and tyramine 77.55 (Cheddar). Max. values recorded in red wine were (mg/l): putrescine 16.2, histamine 7.93, cadaverine 3.02, tyramine 15.64 and phenylethylamine 5.12. Max. values recorded in fish were (mg/ kg): putrescine 6.17 (redfish), histamine 6.58 (redfish), cadaverine 34.3 (redfish) and spermine 24.0 (coley).

**Pechanek, V., G. Blaicher, W. Pfannhauser, and H. Woidich. 1980.** Application of column liquid chromatography (HPLC) to special problems in food chemistry. A laboratory note. *Chromatographia* 13 (7): 421-427.

This survey gives a short account of the applications and design of the column liquid analyser, and HPLC conditions used in the authors' laboratory during the last 5 yr. Liquid chromatography was used for the detn. of amino acids, sugars and biogenic amines in various foods. Special problems, e.g. detn. of patulin in apple juice, and hyoscyamine and scopolamine in canned French beans were also solved by HPLC. [From En summ.] [See FSTA (1981) 13 3A108.].

**Pegg, A. E. 1986.** Recent advances in the biochemistry of polyamines in eukaryotes. *Biochem. J.* 234: 249-262.

**Pemberton, I. J., G. R. Smith, T. D. A. Forbes, and C. M. Hensarling. 1993.** An improved method for extraction and quantification of toxic phenethylamines from Acacia berlandieri. *Journal of Animal Science* 71 (2): 467-470.

A simplified, rapid procedure is described for the extraction and quantification of naturally occurring beta-phenethylamines from A. berlandieri using solid-phase extraction and reversed phase high performance liquid chromatography. Recovery efficiency of 125 µg/ml amino standards averaged 97, 101 and 98% respectively for tyramine, hordenine and N-methyl-beta- phenethylamine.

**Peric M, Buncic S, Smiljanic D, and N. Rajkovic. 1984.** [Enteropathogenic Escherichia coli and histamine formation in meat.]. *Tehnologija Mesa* 25 (3): 66-68.

Histamine formation in meat inoculated with enteropathogenic Escherichia coli was investigated. Samples of comminuted beef, pork and poultry were inoculated with E. coli of the strains 055, 0111 and 0125, at 10-6/g, and incubated for 2 days at 20 degree C or 5 days at 10 degree C. Data are given for counts of E. coli and for histamine formation. Counts were higher after 2 days at 20 degree C than after 5 days at 10 degree C. No histamine formation was obtained at 10 degree C; at 20 degree C, histamine formation was observed in beef (20 mug/g), pork (40 mug/g) and poultry white meat (20 mug/kg), but not poultry dark meat.

**Pfaender, P. 1983.** Lysinoalanine - a toxic compound in processed proteinaceous foods. *World Review of Nutrition and Dietetics* 41: 97-109.

Aspects of lysinoalanine (LAL) considered in this review include alkali-induced formation of LAL, LAL synthesis, detn., toxicological and nutritional implications of LAL in food, and inhibition of LAL formation. Results of LAL assays in a number of home-cooked foods, food ingredients and commercial food preparations are presented. It is concluded that although LAL toxicity in humans remains unclear, inhibition of LAL formation is desirable.

**Pfannhauser, W., and U. Pechanek. 1984.** [Biogenic amines in foods: formation, occurrence, analysis and toxicological evaluation.] Biogene Amine in Lebensmitteln: Bildung, Vorkommen, Analytik und toxikologische Bewertung. *Zeitschrift fuer die Gesamte Hygiene und ihre Grenzgebiete* 30 (2): 66-76.

Aspects considered in this review on biogenic amines (histamine, tyramine, phenylethylamine, diamines, polyamines) include: biosynthesis; degradation; toxicology; occurrence in foods (fish, cheese, wine, meat, raw ripened sausage); formation of nitrosamines; and tolerances etc. for histamine in various countries.

**Pfundstein, B., A. R. Tricker, E. Theobald, B. Spiegelhalder, and R. Preussmann. 1991.** Mean daily intake of primary and secondary amines from foods and beverages in West Germany in 1989-1990. *Food and Chemical Toxicology* 29 (11): 733- 739.

Samples of foods and alcoholic beverages (264) from the German market in 1989-1990 [see preceding abstr.] were analysed for primary and secondary amines. Amines were determined as benzenesulphonyl chloride derivatives by GC with chemiluminescence detection. Mean daily intake of primary amines was calculated to be 29 mg/day for women and 37 mg/day for men. For secondary amines, mean daily intake was 6 mg/day for women and 8 mg/day for men.

**Plowman, J. E., and Close EA. 1988.** An evaluation of a method to differentiate the species of origin of meats on the basis of the contents of anserine, balenine and carnosine in skeletal muscle. *Journal of the Science of Food and Agriculture* 45 (1): 69-78.

The HPLC method proposed by Carnegie [see FSTA (1983) 15 10S1814 and FSTA (1987) 19 1S67] for determining the sp. of origin of meats in cooked products was evaluated. Dipeptides (e.g. anserine, balenine [ophidine] and carnosine) in muscle samples obtained from lambs, pigs, beef cattle, horses, red deer, chickens, cats, rabbits, hares, opossums, wallaby and goat are shown in tables. Meat pies prepared using mixtures of pork and beef (in varying proportions) were also examined. All meat samples were collected in New Zealand (comparisons were made with Australian data). Results suggest that the relative concn. of anserine, balenine and carnosine were characteristic of sp. and could be used as a means of identification. Venison (from red deer) and pork both contained significant levels of balenine which differentiated them from the other spp. being investigated; they were distinguished from each other because their carnosine/anserine ratios differed. This is the first report of significant quantities of balenine in red deer meat. Other distinctions included: sheep from beef; cat from hare, rabbit and chicken; and hare from cat and lamb. However, at the 95% confidence level it was not possible to distinguish between rabbit, chicken, opossum and lamb or lamb and cat. In both pig and horse, anserine concn. were low and difficult to determine accurately; horse meat was readily distinguished from other spp. by very low anserine concn. Application of the method to cooked meat pies indicated that very inaccurate predictions of relative meat proportions, based on dipeptide ratios, would be obtained. Reasons for this and other limitations of the method are discussed.

**Pollack, P. F., O. Koldovsky, and K. Nishioka. 1992.** Polyamines in human and rat milk and in infant formulas. *American Journal of Clinical Nutrition* 56 (2): 371-375.

Using HPLC techniques the presence of putrescine, spermidine, and spermine was verified in human milk and the concentration estimated in samples collected from the first week up to 4 months of lactation. Mean values were (per litre) from 0 to 615 nmol putrescine, from 73 to 3512 nmol spermidine, and from 722 to 4458 nmol spermine. Polyamine concentrations in infant formulae were dependent on the protein source, the particular polyamine, and the protein concentration of the formula. Concentrations of these 3 compounds in rat milk over the first 3 weeks of lactation were higher than in human milk, with spermidine being the polyamine most increased compared with human milk (almost 20-fold higher). An artificial formula used for the rearing of sucking rats contained trace to immeasurable amounts of polyamines. The

study identifies milk as one vehicle for polyamine delivery to the intestinal mucosa of sucking animals.

**Pozo, R. G., and E. S. Saitua. 1988.** Estimation of histamine and its metabolites in fish using HPLC with fluorimetric detection. *Alimentaria* 25 (196): 27-29.

High-performance liquid chromatography with pre-column derivatization with o-phthalaldehyde followed by fluorometric detection was used to estimate histamine in fish. The limit of detection was 0.16 ng histamine and the response was linear over a range of 1 to 2 ng. The average histamine recovery from fortified tuna samples was 91.4 with a standard deviation of 2.14%.

**Ramantanis, S., C. P. Fassbender, and S. Wenzel. 1985.** [Formation of histamine, tyramine and tryptamine in dry sausages.] Untersuchungen zur Bildung von Histamin, Tyramin and Tryptamin in Rohwuersten. *Archiv fuer Lebensmittelhygiene* 36 (1): 9-11.

3 types of dry sausages (Braunschweiger, Bauernmettwurst and Cervelat) fermented by the natural microflora under commercial conditions were examined for biogenic amines during ripening and up to 49 days' storage at 2 degree C, by TLC (all 3 amines) or fluorimetry (histamine). Both methods had a 90% recovery rate, with detection limit 10 mug/g or 0.1 mug/g resp. Results of histamine and tyramine detn., shown graphically and in tables, revealed distinct, mostly continuous, increases during ripening, with mean contents of 3.60-8.23 and greater than 10-93.24 mug/g resp. at the end of ripening. Tyramine showed a significant, continuous rise during storage, while histamine concn. remained constant, suggesting that tyramine concn. could be an indicator of the freshness of these products.

**Rice, S., Eitenmiller, R.R. and Koehler, P.E. 1975.** Histamine and tyramine content of meat products. *J. Milk Food Technol.* 38: 256-258.

A survey was conducted to determine the histamine and tyramine contents of a variety of meat products. Histamine was found in all products at concn. only slightly greater than one would expect from normal physiological amounts found in muscle. Semi-dry sausage products had an average histamine concn. of 3.59 mug/g compared to 2.87 mug/g in dry sausages. Country-cured hams averaged 1.69 mug histamine/g. Emulsion-type products contained slightly less histamine than fermented sausages. Braunschweiger, an exception, contained 3.6 mug histamine/g but would be expected to contain more histamine than other emulsion-type products because of its liver content. Data indicate than histamine is not formed to an appreciable extent in these meat products under normal processing conditions. Detectable amounts of tyramine were found in 71% of dry sausages and in 39% of semi-dry sausages. Tyramine was not detectable in country-cured ham. Average tyramine concn. were 244 and 85.8 mug/g in dry and semi-dry sausages, respectively. The greatest tyramine concn. found in this study was 1237 mug/g in Genoa salami. It is apparent that sufficient tyramine can occur in ripened sausages to be troublesome to tyramine-susceptible individuals.

**Rice, S. L., R. R. Eitenmiller, and P. E. Koehler. 1976.** Biologically active amines in food: a review. *Journal of Milk and Food Technology* 39 (5): 353-358.

Biologically active amines are normal constituents of many foods and have been found in cheese, sauerkraut, wine and putried, aged, or fermented meats. These low mol. wt. organic bases do not represent any hazard to individuals unless large quantities are ingested or natural mechanisms for their catabolism are inhibited or genetically deficient. Tyramine, histamine, and phenethylamine, which can arise from enzymatic decarboxylation of the corresponding amino acids, are strongly vasoactive. Histamine, a capillary dilator, produces hypotensive effects, while tyramine and phenethylamine cause a rise in blood

pressure. Phenethylamine has been implicated in the onset of migraine headache attacks. The occurrence, mechanism of formation, and catabolism of these compounds are reviewed.

**Rodriguez Jerez, J. J., M. T. Mora Ventura, and T. Civera. 1994.** Histamine and fish: an overview. I. Main factors involved. *Industrie Alimentari* 33 (324): 299-307.

This review covers: toxicity of histamine in foods; histamine in fish and other sea foods; fish species commonly implicated; bacteria responsible for histamine formation; bacterial formation of putrescine and cadaverine; effects of histidine concn.; and effects of pH, carbohydrates, vitamins, coenzymes, NaCl, and O2 on histamine formation.

**Rodriguez Jerez, J. J., M. T. Mora Ventura, and T. Civera. 1994.** Histamine and fish: an overview of a recent concern. II. Relationship between fish spoilage and histamine formation. *Industrie Alimentari* 33 (325): 393-399.

This second part of this review on histamine and fish [see preceding abstr. for previous part] covers: the relationship between spoilage and histamine formation in fish; toxicity of histamine; synergistic and antagonistic factors; metabolism of histamine; determination of histamine in fish; and isolation and identification of histamine-forming bacteria.

**Romain, N., G. Dandrifosse, F. Jeusette, and P. Forget. 1992.** Polyamine concentration in rat milk and food, human milk, and infant formulas. *Pediatric Research* 32 (1): 58-63.

The polyamine concentration in rat milk and food, human milk and infant formulae was estimated by HPLC. In rat milk, the concentration of putrescine and spermine was low (generally under 2.5 nmol/ml for putrescine and under 1 nmol/ml for spermine). The spermidine concentration was higher and seemed to increase during lactation. Rat food was richer in polyamines than rat milk (about 150 times for putrescine and spermine, about 30 times for spermidine). It is suggested that polyamines contained in rat food could play an important role in postnatal maturation of the rat intestine. The polyamine concentration of human milk was measured from 60 different mothers during a period extending from the 1st week to the 6th month of lactation. Great variation was observed. During the 1st month of lactation putrescine concentration generally varied little (from 1 to 3 nmol/ml), spermine and spermidine concentrations showed a similar pattern (the highest values appeared at the end of the 1st week of suckling). After the 4th month of lactation, putrescine concentration increased slightly, whereas spermine and spermidine concentration stayed almost stable. The concentrations of polyamines in 18 dried milks for infants were estimated. Spermine and spermidine contents were lower than those in human milk. A protective effect of spermine or spermidine against alimentary allergies is suggested.

**Satrulee, R., C. Jaengsaqang, and P. Srisuk. 1990.** Histamine in scombroid products of Thailand. *Pages 121-125 in FAO Fisheries Report No. 401, suppl. from the 7th Session of the Indo-Pacific Fishery Commission Working Party on Fish Technology and Marketing.*

Histamine content of 607 samples of mackerel, sardine, tuna, bonito, yellow fin and skipjack packed in oil and in brine in chunks and solid pack styles produced in Thailand in 1987 was estimated fluorimetrically. 100% of mackerel, 95% of sardine, 100% of solid style and 95% of chunk style of tuna contained histamine <50 mg/kg. Only 2% of chunk style tuna had histamine >100 mg/kg.

**Sayem-El-Daher, N., Simard, R.E. and Fillion, J. 1984.** Changes in the amine content of ground beef during

storage and processing. *Lebensmittel Wissenschaft Und Technologie* 17: 319-323.

Evolution of 7 biogenic amines e.g., 1,3 diaminopropane (DAP) histamine (HA), putrescine (PUT), cadaverine (CD), spermine (SM), spermidine (SD) and tyramine (TA), as a function of storage time and temp. and of cooking methods was studied. Amines were extracted with 0.6N HClO4 and analysed by ion exchange column chromatography using a Technicon amino acid analyzer and a C4 cation-exchange resin column. Concn. of the major amines in cooked and uncooked samples stored at 4 degree , 7 degree and 10 degree C for 0-12 days (readings taken at 48 h intervals) are tabulated. Typical ranges for amines after 12 days storage (varying with temp.) are DAP 1.1-2.5, PUT 7.4-36.8, HA 3.2-2.9, CD 0- 10.8, SM 33.1-44.6, SD 11.3-17.7 and TA 1.2-3.3 mg/100 g of raw ground beef. All concn. (except HA which was unaffected by storage conditions) were positively correlated with storage time and temp. All were unaffected by cooking (except SM concn., which decreased). Results confirmed the usefulness of amines as indicators of beef quality.

**Sayem-El-Daher, N., Simard, R.E. and L'Heureux, L. 1983.** Determination of mono-, di- and polyamines in foods using a single-column amino acid auto-analyzer. *J. Chromatog.* 256: 313-321.

A fully automated, rapid and sensitive method was developed to analyse 14 biogenic amines in food. Using a Technicon C4 ion-exchange resin column (20 m x 0.5 cm), adapted to an automatic Technicon TSM amino acid analyser, the following amines were separated and quantified: adrenaline, noradrenaline, 1,3-diaminopropane, putrescine, cadaverine, histamine, spermidine, dopamine, spermine, agmatine, tyramine, serotonin, phenethylamine and tryptamine. Five buffers were required to elute the amines using a gradient of pH from 5.6 to 12.7; the column temp. was maintained at 65 degree C. The method was also assayed on ground beef, cheese and wine samples. Amines from cheese and ground beef samples were extracted with 0.6M perchloric acid. No extraction of wine samples was necessary.

**Schmitt, R. E., Haas J, and Amado R. 1988.** [Determination of biogenic amines by reversed-phase HPLC for monitoring microbial spoilage of poultry.] Bestimmung von biogenen Aminen mit RP-HPLC zur Erfassung des mikrobiellen Verderbs von Schlachtgefluegel. *Zeitschrift fuer Lebensmittel Untersuchung und Forschung* 187 (2): 121-124.

A rapid method, incorporating a simple sample preparation technique and reversed-phase HPLC, is described for detn. of biogenic amines in poultry skin as an indicator of microbial spoilage. Poultry skin samples are homogenized and extracted with 0.6M perchloric acid under mild conditions (shaking). Extracts are derivatized with dansyl chloride and amines are separated by HPLC on a Hypersil ODS (RP-18) column by gradient elution (increasing concn. of methanol in an acetonitrile/0.02M acetic acid/methanol system). Quantitation of spermine and spermidine requires additional cleanup of extract on Amberlite CG 50 prior to dansylation. 8 amines can be separated within 7 min. Recoveries were 82-96% and limits of detection were 0.2-0.5 mug/ g skin. 6 amines were found in poultry skin, 4 of which, putrescine, cadaverine, histamine and tyramine were indicators of spoilage, especially the 1st 2 as they are not detectable in skin of fresh poultry carcasses, and increase in concn. markedly with onset of spoilage and increase in total colony counts beyond Z 10-5 cfu/cm-2.

**Schmitt, R. E., and Schmidt Lorenz W. 1992.** Formation of ammonia and amines during microbial spoilage of refrigerated broilers. *Lebensmittel Wissenschaft und Technologie* 25 (1): 6- 10.

Formation of amines and ammonia during microbial spoilage of broilers, stored unpacked in a desiccator and in the original film packaging at 4 degree C was investigated. In the pre- spoilage stage, concn. of ammonia in the skin increased slightly. There was a rapid rise in the concn. of ammonia with the onset of spoilage; final concn. reached 67.3 and 51.8 mug/g skin in unpacked and packed carcasses, resp. Putrescine, cadaverine, histamine and tyramine were detectable after 4 and 6 days, resp. Diamine concn.

rose rapidly with the onset of spoilage. For both types of storage, cadaverine was the major biogenic amine detected; spermidine and spermine showed no changes during storage. It is concluded that putrescine and cadaverine detectable from colony counts of 10-5 cfu/cm-2 could be used as indicators for the onset of spoilage; detn. of ammonia only has limited value as a quality index for poultry carcasses.

**Schmitt, R.E. and Schmidt-Lorenz, W. 1992.** Degradation of amino acids and protein changes during microbial spoilage of chilled unpacked and packed chicken carcasses. *Lebensmittel Wissenschaft Und Technologie* 25: 11-20.

Bacterial breakdown of proteins and amino acids during spoilage of chicken carcasses, stored unpacked in desiccators and in the original polyethylene bags at 4 degree C for 20 days, was investigated. After 6 and 8 days, spoilage onset was noticeable in unpacked and packed carcasses, resp.; pseudomonads were predominant in both cases after 4 days. Amino acid composition after acid hydrolysis of proteins remained constant during the whole storage period for the unpackaged carcasses; free amino acids increased in proportion to colony counts. In packaged chickens, there was an initial rise in free amino acids at the onset of storage; before the onset of spoilage, free citrulline, which was not detected in the skins of freshly slaughtered chickens, increased. Therefore, citrulline is proposed as a quality index. Prior to the onset of spoilage, changes in the proteins could not be detected.

**Schneider, R., F. Kreienbring, G. Bolduan, and M. Beck. 1989.** Biogenic amines in the digesta of pigs. *Archives of Animal Nutrition* 39 (12): 1021-1029.

Amine concentrations in the digesta of weaned and finishing pigs, weighing 12-14 and 121 kg, were estimated. Diets were isonitrogenous (weaned pigs) and isoenergetic (finishing pigs) and contained only small amounts of putrescine. Equal proportions of histamine, tyramine, putrescine and cadaverine were found in the stomach, while most cadaverine was found in the colon. Weaned pigs given a diet with 24 or 18% crude protein had concentrations of amines 4.2, 10.2 and 9.6, and 0, 5.5 and 4.9 mmol/kg DM in stomach, small and large intestine, respectively. Digesta of finishing pigs contained amines 2.1 to 8.7, 1.0 to 4.8 and 0.2 to 2.2 mmol/kg DM in jejunum, caecum and colon, respectively. Increasing crude fibre content and bactericides in feeds did not significantly decrease concentrations of amines in all gut sections.

**Schuber, F. 1989.** Influence of polyamines on membrane functions. *Biochem. J.* 260: 1-10.

**Shalaby, A. R. 1994.** Separation, identification and estimation of biogenic amines in foods by thin-layer chromatography. *Food Chemistry* 49 (3): 305-310.

A method for the separation, identification and estimation of 8 biogenic amines (histamine, cadaverine, putrescine, phenylethylamine, tyramine, tryptamine, spermidine and spermine) using silica gel TLC and spectrophotofluorometry is described. The complete resolution of the dansylated derivatives of amines could not be achieved by 1-dimensional TLC using any of 12 solvent systems examined. However, the derivatives could be well separated by 2-dimensional TLC in which the 1st development system was benzene/triethylamine/acetone (10:1:2, v/v/v), while in the 2nd direction the development system was benzene: triethylamine (5:1 v/v). The Rf values and the fluorescent colours of the amine dansyl derivatives aided their identification. The separated fluorescent derivatives were extracted with acetonitrile and estimated spectrophotofluorometrically. Results indicated that the method is sensitive and precise, where the relative s.d. was less less than 10%. The method was applied to 10 dry sausage samples and 10 fish samples. Dry sausage contained the 8 biogenic amines with an average concn. similar to that which has been previously reported for sausage or meat products. Fish samples were free from tyramine, tryptamine and phenylethylamine. The other amines were found to be variable in their concn.

**Shalaby, A. R. 1993.** Survey on biogenic amines in Egyptian foods: sausage. *Journal of the Science of Food and Agriculture* 62 (3): 291-293.

The biogenic amine content of 50 Egyptian sausages obtained from a market in Cairo was estimated by high performance liquid chromatography after extraction in 5% trichloracetic acid. Histamine was present in 46% of the sausages at about 5.25 mg/kg, while putrescine and cadaverine were present in 96 and 94% of the sausages respectively. The corresponding average concentrations were 38.62 and 19.20 mg/kg. Tyramine was present in 78% of the sausages and tryptamine was present in 68% of the tested sausages, while 18% of the tested sausages contained phenylethylamine. The average concentrations of 19.25, 12.70 and 33.25 mg/kg were obtained for tyramine, tryptamine and phenylethylamine, respectively. The polyamines, spermine and spermidine, were present in 54 and 44% of the sausages, with an average of 1.75 and 2.30 mg/kg, respectively.

**Sieber, R., and P. Lavanchy. 1990.** Biogenic amines in dairy products and cheese. *Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene* 81 (1): 82-105.

This is a review of values reported in the literature for histamine, tyramine, phenethylamine, tryptamine, cadaverine and putrescine in milk, cultured milks, dried milk, cream and especially cheese.

**Simon Sarkadi, L., and W. H. Holzapfel. 1994.** Determination of biogenic amines in leafy vegetables by amino acid analyser. *Zeitschrift fur Lebensmittel Untersuchung und Forschung* 198 (3): 230-233.

An ion exchange chromatographic method for estimating biogenic amines in salad vegetables (chinese cabbage, endive, iceberg lettuce and radicchio [red chicory]) is described. Separation was by potassium cation exchange column with 3 buffer systems. Amines separated and quantified in chinese cabbage, endive, iceberg lettuce and radicchio were putrescine, histamine, cadaverine, spermidine, agmatine, spermine and tyramine. Amines were extracted with 10% trichloroacetic acid; total concentration of biogenic amines was 14-20 µg/g fresh matter. Spermidine was the major polyamine detected at 7-15 µg/g fresh matter.

**Skog, K., Johansson, M. and Jaegerstad, M. 1994.** A review of mutagenic heterocyclic amines. *Scand. J. Nutr.* 38: 30-33.

Properties of heterocyclic aromatic amines (HA) are reviewed. HA are produced from amino acids or proteins after heating at high temp. ( greater than 300 degree C). At normal cooking temp., imidazo-containing HA are produced via the Maillard reaction. They are known to be carcinogenic to animals, producing tumours in the liver, gastrointestinal tract and mammary glands. Overall daily consumption is estimated to be between 1 and 20 mug. HA have been isolated and identified in cooked meat and fish products, including gravies and extracts; they have also been quantified in beer and wine. [From En summ.].

**Slemr, J., and K. Beyermann. 1985.** Concentration profiles of diamines in fresh and aerobically stored pork and beef. *Journal of Agricultural and Food Chemistry* 33 (3): 336-339.

Putrescine, cadaverine and histamine were estimated in commercial fresh pork and beef stored aerobically at low temperatures. Chemical analyses were made in parallel with estimations of total bacterial numbers and sensory assessments. Meat samples were grouped into 3 quality classes according to bacterial

numbers and sensory quality, and cutoff values for the combined diamine concentration were proposed. The diamine content made it possible to distinguish between classes 1 and 2 of pork with a maximum disagreement of 14%.

**Slump, P. 1978.** Lysinoalanine in alkali-treated proteins and factors influencing its biological activity. *Annales de la Nutrition et de l'Alimentation* 32 (2/3): 271-279.

In vitro studies were carried out on the biological activity of lysinoalanine (LAL), formed during alkali treatment of proteins by combination of dehydroalanyl (from cystyl or seryl residues) with lysyl residues. Samples of alkali treated (AT) soya protein, lactalbumin and casein were subjected to enzymic hydrolysis. A combination of pepsin-pancreatin-pig intestinal mucosa or pepsin- pronase-prolidase-aminopeptidase released 3% of LAL in AT soya protein (containing 1.3 g LAL/16 g N), less than0.2% in AT casein (containing 5.5 g LAL/16 g N) and 0.5% from mildly AT casein (1.0 g LAL/16 g N, first enzyme combination only). When only pepsin and pancreatin were used, the hydrolysates from AT soy-bean protein, lactalbumin and casein contained LAL almost exclusively as peptides. The % of LAL in small peptides increased with decreasing LAL contents (e.g. from 3% at 5.5 g LAL/16 g N to 69% at 1.0 g LAL/16 g N). These results, together with feeding studies, suggest that the activity of protein-bound LAL may increase with decreasing severity of alkali treatment. In view of the probable amounts in the diet, risks from LAL in human foods are thought to be low. [See FSTA (1979) 11 5A346.].

**Smith, T. K. 1990.** Effect of dietary putrescine on whole body growth and polyamine metabolism. *Proc. Soc. Exp. Biol. Med.* 194 (4): 332-336.

For 14 days 96 chickens were given diets with purified crystalline amino acid without or with 0.2, 0.4, 0.6, 0.8 or 1.0% purified putrescine. Putrescine 0.2% increased growth rate beyond that of controls whereas further supplements reduced growth and were toxic when 0.8 or 1.0% was given. Hepatic and muscle concentrations of ornithine increased with dietary putrescine whereas the effect in kidney was much less. Putrescine concentrations in liver, kidney and muscle increased when 0.4% putrescine or more was given. This effect was particularly obvious in muscle in which there were also increases in the concentrations of spermidine and spermine. In a subsequent similar experiment, putrescine was given at 0.1, 0.2, 0.3, 0.4 or 0.5% to determine the effect on the activities of the key enzymes regulating polyamine synthesis. Even 0.1% putrescine caused a rapid reduction in hepatic ornithine decarboxylase activity whereas S-adenosylmethionine decarboxylase and arginase activities were not influenced by diet.

**Smith, T.A. 1985.** Polyamines. *Ann. Rev. Plant Physiol.* 36: 117-143.

A review of polyamine biosynthesis, catabolism, derivatives and conjugates in plants, and of growth regulation by polyamines, membranes and senescence as affected by polyamines, interactions with growth substances, and the response of polyamines to stress. Work on field and horticultural crops is quoted.

**Smith, T. A. 1981.** Amines in food. *Food Chemistry;* 6 (3): 169-200.

This review discusses the role of aromatic amines and contains sections on amines formed in various foods by bacterial activity, and amines in plant products. The structures of some amines and related products found in foods are discussed, and published data on amines in cheese, yeast extract, alcoholic drinks and meat products, histamine and methylamines in fish, aliphatic monoamines, phenethylamines, tryptamines and histamine in edible plant products, and amines in banana pulp are tabulated.

**Smith, T. A. 1977.** Phenethylamine and related compounds in plants. *Phytochemistry* 16 (1): 9-18.

A comprehensive and up-to-date listing is provided of the distribution of phenethylamines in the Plant Kingdom. Such a listing is of importance because of their considerable physiological activity in higher animals e.g. they may precipitate migraines.

**Smith, T. A. 1977.** Tryptamine and related compounds in plants. *Phytochemistry* 16 (2): 171-175.

Ingestion of large amounts of the tryptamines may be harmful to humans and to some animals. The occurrence of the tryptamines and related compounds in fungi and higher plants is listed on a taxonomic basis. Several of these amines have considerable physiological activity in higher animals.

**Smith, T. A. 1975.** Recent advances in the biochemistry of plant amines. *Phytochemistry* 14 (4): 865-890.

This review of the biochemistry of plant amines (including those present in food plants) covers simple aliphatic amines, di- and polyamines, amine oxidase and di- and polyamine conjugates.

**Smith, T.A. 1970.** Putrescine, spermidine and spermine in higher plants. *Phytochemistry* 9: 1479-1486.

In view of the importance of polyamines in activating protein synthesis, putrescine and the 2 polyamines spermidine and spermine were quantitatively determined in the leaves of wheat, strawberry, apple, tomato, marrow and spinach, roots of pea and fruits of apple. In tissues of maize, barley, radish, pea, blackcurrant and tobacco grown in K-deficient conditions, comparison with normal plants showed a 16.6 fold mean increase in concn. of putrescine, 1.9 fold of spermidine and 1.2 fold of spermine. This is compatible with consecutive conversion of putrescine into spermidine and spermine.

**Smith, J. S., Kenney P.B., C. L. Kastner, and M. M. Moore. 1993.** Biogenic amine formation in fresh vacuum-packaged beef during storage at 1 degree for 120 days. *Journal of Food Protection* 56 (6): 497-500.

Bacterial proteolysis and decarboxylation, which may occur in vacuum-packaged beef products, can release pressor amines, such as tyramine and histamine. These amines can be toxic when ingested by individuals taking monoamine oxidase-inhibiting drugs. Effect of carcass decontamination on bacterial growth and biogenic amine production in vacuum-packaged subprimals was determined. Cattle carcasses were treated with 200 p.p.m. chlorine or 3% lactic acid sprays, fabricated, vacuum packaged and stored at 1 degree C. Samples were evaluated for up to 120 days for amine concn., total aerobic counts and lactic acid bacteria. Of the amines monitored, only tyramine was consistently detected. Significant levels of tyramine were detected starting at day 20 of storage in all treatments and controls. By day 60, levels had increased to approx. 50 mug/g and continued to increase to approx. 180 mug/g after 120 days storage. Tryptamine was detected in some samples after 60 days storage, but levels were variable and did not follow a trend. Initial aerobic plate counts ranged from 10 to 200 cfu/cm-2, whereas lactic acid bacteria counts were from 6 to 46 cfu/cm-2. Bacterial numbers increased exponentially until around day 60, when they levelled off at between 10-6 and 10-7 cfu/cm-2, with no differences between any of the treatments and/or controls. Although vacuum- packaged beef was sensorially acceptable up to day 60 (day 90 for some samples), it could pose some risk to individuals sensitive to biogenic amines if the product is stored at ≥1 degree C for ≥60 days.

**Sousadias, M. G. and Smith, T. K. 1995.** Toxicity and growth-promoting potential of spermine when fed to chicks. *J. Anim. Sci.* 73: 2375-2381.

Previous studies have shown that the feeding of putrescine, a biogenic amine and the precursor of the mammalian polyamines, can promote whole-body growth of chicks. The current study was undertaken to determine the effect of spermine, also a biogenic amine and the most cationic of the polyamines, under similar conditions. In Exp. 1, 120 week-old chicks were fed purified crystalline amino acid-based diets containing 0, .2, .4, .6, .8, or 1.0% spermine for 14 d. Spermine proved highly toxic and growth rates were reduced compared with controls when even .2% was fed. In Exp. 2, chicks were fed 0, .0375, .0750, or .1000% spermine. These concentrations proved less toxic than those used in Exp. 1. Supplemental dietary cysteine was then provided at 0, .3, .6, and .9% together with 0, .025, .050, or .400% spermine (Exp. 3) because depletion of cellular glutathione has been suggested as contributing to spermine's toxicity. Even high levels of cysteine supplementation did not overcome spermine's toxicity. Subsequent dietary provision of L-2-oxothiazolidine-4-carboxylic acid (OTC, Exp. 4), a cysteine prodrug, showed that depletion of cellular glutathione was not likely a cause of spermine toxicosis. A trend toward increased weight gain and feed efficiency was observed when low concentrations of spermine were fed. It was concluded, however, that dietary spermine was more toxic to chicks than was previously seen for putrescine, that any growth-promoting effects of dietary spermine are small, and that supplements of dietary cysteine or OTC are unlikely to increase these effects by overcoming spermine toxicosis.

**Srivastava, A. K., M. Sabir, and J. K. Malik. 1986.** Studies on the interaction of histamine with blood serum from Gallus domesticus (White Leghorn). *Acta Veterinaria* 36 (2/3): 133-139.

The effect of avian serum proteins derived from healthy or parasitized fowls on the spasm-inducing action of histamine on isolated guinea-pig ileum was studied. Twenty birds were healthy and uninfected, 10 were infected with coccidia, 8 with ascaridia and 24 experimentally infected with the eye fluke, Philophthalmus gralli. The standard spasmogenic dose of histamine selected for this study was 0.1 µg per 10 ml of bath fluid. Based on the height of contraction, serum from healthy birds inhibited the histamine-induced response by about 33%. Serum from fowls harbouring ascaridia or coccidia potentiated the response by an average of 188 and 90%, respectively. Serum from the experimentally infected fowls potentiated the contraction by averages of 11, 23 and 63% at 15, 20 and 25 days after infection, respectively. This inhibitory property of serum proteins on the spasmogenic effect of histamine, a property termed histaminopexy, may be carried by albumin.

**Staruszkiewicz, W. F. J., and J. F. Bond. 1981.** Gas chromatographic determination of cadaverine, putrescine, and histamine in foods. *J. Assoc. Offic. Anal. Chemists* 64 (3): 584-591.

A GLC procedure for quantitative detn. of the diamines putrescine and cadaverine was developed, using their perfluoropropionyl derivatives. The amines were extracted from foods with methanol; an internal standard, hexanediamine, was added and a dry residue of their hydrochloride salts was prepared. The salts were derivatized with perfluoropropionic anhydride by heating for 30 min at 50 degree C. The reaction mixture was separated on an alumina column to remove excess reagent, and the derivatives were eluted with a solution of 30% ethyl acetate in toluene. GLC separations were performed on a 3% OV-225 column held at 180 degree C. The retention times were 4.3, 5.7, and 7.0 min for the derivatives for putrescine, cadaverine, and the internal standard, resp. Less than 1 mum diamine/g tissue could be quantitated, using either an electron capture detector or a nitrogen-specific detector. The procedure was applied to cheese and a variety of fishery products. An increase in the diamines correlated with the presence of decomposition in some of the products. A collaborative study of the method is planned.

**Seiler, N. and Knodgen, B. 1980.** High performance liquid chromatographic procedure for the simultaneous determination of the natural polyamines and their mono acetyl derivatives. *J. Chromatog.* 221: 227-235.

The separation of the natural polyamines and their monoacetyl derivatives by high-performance reversed-phase liquid chromatography is reported. Octane sulfonate was used to form ion pairs with the polycations

and the .omega.-phthalaldehyde method for post-column derivatization. The method allows polyamine and acetylspermidine determinations directly from [rat] tissue extracts and body fluids without pre-purification.

**Sen, N.P. 1969.** Analysis and significance of tyramine in foods. *J. Food Sci.* 34:22-26.

Tyramine levels in different varieties of cheeses, alcoholic drinks, and other foods were determined. Many of the food samples, when analysed by an existing fluorometric method, contained large amounts (less than2.2 mg/g) of tyramine. Synephrine and octopamine, although rarely present in food, interfered with the fluorometric analysis. A gas chromatographic method was developed in which tyramine was extracted from foods and then analysed as its trifluoroacetyl derivative using an electron capture detector. The gas chromatographic method was more sensitive and specific than the fluorometric method, and permitted detection of 0.1-0.2 ng tyramine even in the presence of synephrine and octopamine.

**Sternberg M, C. Y. Kim, and Schwende FJ. 1975.** Lysinoalanine: presence in foods and food ingredients. *Science* 190 (4218): 992-994.

Lysinoalanine (LAL), N–e-6-3-(DL-2-amino-2-carboxyethyl)-L- lysine, an unusual amino acid implicated as a renal toxic factor in rats, has now been shown to be generated in various proteins heated under nonalkaline, as well as alkaline conditions. Tabulated results of assays of foods and ingredients show LAL contents (mug LAL/g protein) of: in home-cooked foods - frankfurter, 0 (uncooked)-170; chicken thigh, 0 (raw)-200; sirlon steak pan scrapings, 130; egg white, 0 (fresh)-1100: commercial food preparations - corn chips, 390; pretzels, 500; infant milk formulae, 150-640; evaporated milk, 590-860; evaporated skim-milk, 520; condensed milk, 360-540; simulated cheese, 1070: food ingredients - dried egg white solids, 160-1820; calcium caseinate, 370-1000; sodium caseinate, 430-6900; acid casein, 70-190; masa harina, 480; hydrolysed vegetable protein, 40-500; whipping agent, 6500-50 000; soy protein isolate, 0-370; and yeast extract, 120. Soy globulin, ovalbumin, lysozyme, casein and bovine serum albumin all formed variable amounts of LAL when heated under non-alkaline conditions at ranges of pH, temp. and time commonly used. The presence of LAL in proteins may partly explain the reduction of nutritive value by heating.

**Stevanato R, Mondovi B, Sabatini S, and A. Rigo. 1990.** Spectrophotometric assay for total polyamines by immobilized amine oxidases. *Analytical Chimica Acta* 237 (2): 391-397.

Immobilized bovine plasma and pig kidney amine oxidases coupled with the hydrogen peroxide-peroxidase system are proposed for the detection of di- and polyamines in biological samples. The measurements are carried out spectrophotometrically with a flow reactor filled with the enzymes immobilized on Sepharose. [Polyamines are determined by spectrophotometric measurement of $H_2O_2$ produced in their deaminative oxidation.] The simplicity of the procedure and the short analysis time are the main advantages. The kinetic characteristics of the immobilized system, amine oxidases and peroxidase, are also reported. This method, which appears useful for routine analyses, permits the detn. of polyamines in tissue and food homogenates and isolated cells. [Polyamine concn. given for sardine and ham are (mumol/g fresh wt.): spermine, 0.42-0.63 and 0.62-1.50, resp., and putrescine, 0.28-0.33 and 0.10-0.15, resp. Polyamine concn. were measured in sardine stored in closed vials at -30, 4 or 22 degree C for 5 days. Storage at 4 degree C caused a strong increase in putrescine content, from 0.31 to 1.2 mumol/g; after 5 days at 22 degree C, putrescine content was 1.7 mumol/g. Spermine was not affected by storage at -30 or 4 degree C, but increased from 0.48 to 1.00 mumol/g during storage at 22 degree C.] [From En summ.].

**Stratton, J. E., R. W. Hutkins, and S. L. Taylor. 1991.** Biogenic amines in cheese and other fermented foods: a review. *Journal of Food Protection* 54 (6): 460-470.

Biogenic amines (particularly histamine) in foods are reviewed under the following headings: Clinical aspects and toxicology (symptomology, constraints to surveillance, histamine metabolism toxicity and role of potentiators, putrefactive amines, pharmaceutical agents); Fermented foods containing histamine (cheese, formation of other biogenic amines in cheese, fermented beverages, fermented dry sausage, fermented vegetables, miso, soy sauce and related foods, fermented fish products); Formation of histamine and its control (histidine decarboxylase, histamine- producing bacteria, control of histamine formation); Analysis of histamine (AOAC procedure, rapid methods, detn. of histamine- producing bacteria); and Histamine regulation.

**Straub, B. W., P. S. Tichaczek, M. Kicherer, and W. P. Hammes. 1994.** Formation of tyramine by Lactobacillus curvatus LTH 972. *Zeitschrift fuer Lebensmittel Untersuchung und Forschung* 199 (1): 9-12.

Lactobacillus curvatus is used in the food industry for the manufacture of fermented sausages. A strain of L. curvatus, LTH 972, which synthesizes tyramine was isolated from fermented sausages. Factors influencing synthesis of tyramine by L. curvatus LTH 972 were investigated in a liquid culture supplemented with tyrosine. Highest concn. of tyramine (up to 201 mg/l) were formed at 30 degree C, pH 5.2, and aw 0.97. At lower temp. and at higher pH and aw values, the reaction slowed down but was still clearly detectable. Glucose, nitrate and nitrite, at concn. used in sausage fermentation, had no effect on tyramine formation. L. curvatus LTH 972 was able to form tyramine from tyrosine-containing di- and tri- peptides in phosphate buffer. In proteinaceous substrates, increased formation of tyramine by L. curvatus LTH 972 cannot be excluded when ongoing proteolysis creates precursors, e.g. in the presence of proteolytic microorganisms. [From En summ.].

**Stuart, B. P., Cole, R. J., Waller, E. R. and Vesonder, R. E. 1986.** Proventricular hyperplasia (malabsorption syndrome) in broiler chickens. J. Envir. Pathol. Toxicol. Oncology 6: 369-385.

A syndrome has occurred in broilers over the past several years in widespread localities, including Georgia, Arkansas, and Texas. Poor feed conversion and delayed marketing are the principle clinical features. Lesions consist of enlargement of the proventriculus, gizzard erosion and dilatation, and decreased spleen and bursa size. Trichothecenes were demonstrated in the rations sampled at several broiler facilities. The clinical features and gross and microscopic changes observed in the field syndrome were duplicated by feeding chicks multiple combinations of a trichothecene mycotoxin (T-2 toxin) with histamines and diamines for 6 to 8 weeks.

**Studer, A., and H. Traitler. 1982.** Quantitative HPTLC determination of 5-hydroxytryptamides of carboxylic acids and tryptamines in food products. *Journal of High Resolution Chromatography and Chromatography Communications* 5 (10): 581- 582.

An HPTLC method is described for detn. of biogenic amines in food products. It was applied to detect cocoa shell impurities in commercial cocoa powders via the 5-hydroxy tryptamide content (2 p.p.m. in beans, 75 p.p.m. in shells). Of 4 cocoa powders analysed, 2 contained no shell (tryptamide contents 1.4 and 2.1 p.p.m. resp.), and 2 contained small amounts of shell impurities (tryptamide content 6.1 p.p.m., i.e. 5% shell, and 4.3 p.p.m., i.e. 3% shell impurities, resp.).

**Sun Pan, B., and D. James. 1985.** Histamine in marine products: production by bacteria, measurement and prediction of formation. FAO Fisheries Technical Paper 62

This document contains the proceedings of a workshop on Histamine in Marine Products held in Hobart, Australia in Feb. 1984. The proceedings summarize the role of histamine as an indicator of spoilage in

scombroid fish, methods of analysis, descriptions of histamine-producing bacteria and the properties of their decarboxylating enzymes. Information is given on prediction of histamine production by using mathematical models relating spoilage to storage temp., combined with the effects of icing, delayed icing, gutting, canning and other preservation methods. (Notes on safe handling of pathogenic anaerobes, e.g. Clostridium botulinum are appended).

**Sun Pan, B., and J. M. Kuo. 1983.** Effect of visceral proteinase on histamine formation in mackerel. *Proc. 6th International Congress of Food Science and Technology* 2: 54.

Two trypsin-like proteinases were isolated from mackerel viscera with pH optima of 9.0 and 7.6 and temp. optima of 45 degree and 55 degree C. Both were stable at less than 50 degree C and neutral to slightly alkaline pH, being inactivated at greater than 80 degree C or at acidic pH. Treatment of mackerel muscle homogenate with the enzymes increased free histidine and histamine, the latter by 120 p.p.m. after 8 days at 4 degree C, by 500 p.p.m. after 24 h at 28 degree C, and by 205 p.p.m. after 4 h at 37 degree C. Histamine and free histidine contents were higher in ungutted than in gutted mackerel. [See FSTA (1984) 16 9A640.].

**Suzuki, S., K. Kobayashi, J. Noda, T. Suzuki, and K. Takama. 1990.** Simultaneous determination of biogenic amines by reversed- phase high-performance liquid chromatography. *Journal of Chromatography* 508 (1): 225-228.

The combination of hexanesulphonate for ion pairing and sodium perchlorate as the separation buffer in the elution solvent enabled the simultaneous micro-estimation of the major biogenic amines on a high-performance liquid chromatography column in a relatively short elution time (35 min).

**Tabor, C. W. and Tabor, H. 1984.** Polyamines. *Ann. Rev. Biochem.* 53: 749-790.

**Taylor, S.L., Leatherwood, M. and Lieber, E.R. 1978.** Histamine in sauerkraut. *J. Food Sci.* 43: 1030-1032.

A survey of 50 samples of sauerkraut obtained at the retail level revealed an average histamine content of 5.06 mg/100 g. The histamine content ranged from 0.91 mg/100 g to 13.0 mg/100 g. Such histamine levels are considerably lower than the level of 100 mg/100 g which has been associated with outbreaks of food poisoning. On the basis of this survey, commercially available sauerkraut should be considered a low risk product for the development of symptoms of histamine toxicity.

**Takeba, K., F. Murakami, M. Matsumoto, and H. Nakazawa. 1990.** Analysis of tyramine in cheese by high performance liquid chromatography. *Journal of the Food Hygienic Society of Japan* 31 (2): 137-141.

A simple, rapid analytical method was developed for the determination of tyramine in cheese using HPLC with a fluorescence detector (FL). The chromatography was performed on a LiChrosorb RP-Select B column (7 µm, 4 X 250 mm) with a mobile phase consisting of a mixture of 0.01 M phosphate buffer (pH 7.5) and acetonitrile (9:1 v/v), and pumped at a flow rate of 1 ml/min. Fluorometric detection was achieved with an emission wavelength of 305 nm and excitation wavelength of 225 nm. Tyramine was extracted from cheese with 5% perchloric acid followed by clean-up on a C18 cartridge. Av. recoveries from cheeses spiked with tyramine at 1 and 10 µg/g were 98.1 and 97.2% resp. Detection limit of tyramine was 0.25 µg/g. Tyramine was contained in 88% of 336 imported cheeses, 48% of 127 domestic cheeses and 100% of 62 domestic processed cheeses; av. concn. was 195.6, 37.6 and 19.0 µg/g resp.

**Takeda Y, Abe F, and K. Samejima. 1982.** Spermidine and spermine contents in foodstuffs. *Journal of Hygienic Chemistry [Eisei Kagaku]* 28 (5): 279-281.

Concn. of spermidine and spermine, possible precursors of the N- nitrosamines, in uncooked foods were fluorometrically determined by TLC. Levels of the polyamines were determined in 6 kinds of meat and poultry, 10 kinds of fish and shellfish, and 9 kinds of vegetables and fruits. More than 100 nmol/g of spermidine and/or spermine was found in meat, poultry, oyster, cod roe, and green vegetables.

**Tamada, H., R. Nezu, I. Imamura, Y. Matsuo, Y. Takagi, S. Kamata, and A. Okada. 1992.** The dipeptide alanyl-glutamine prevents intestinal mucosal atrophy in parenterally fed rats. *Journal of Parenteral and Enteral Nutrition* 16 (2): 110-116.

Whether the addition of alanyl-glutamine (Ala-Gln) can prevent intestinal mucosal atrophy induced by standard solution of total parenteral nutrition (S-TPN) was investigated in 41 male Sprague-Dawley rats (250 g) that were randomly divided into 4 groups: group 1 was killed after overnight fasting; group 2 received S-TPN. The other groups received S-TPN supplemented with amino acids other than glutamine (group 3) or supplemented with Ala-Gln 2 g/100 ml (group 4); both solutions were isoenergetic and isonitrogenous. After 1 week of TPN the rats were killed, and the duodenum, proximal jejunum, mid-small bowel, and distal ileum were obtained for morphologic and functional analysis. Weight gain did not differ significantly among groups, and there was no difference in nitrogen balance between groups 3 and 4. Serum glutamine in group 4 (102.8 ± 13.3 µmol 100 ml) was increased ($P<0.05$) compared with groups 1, 2 and 3 (66.2 ± 3.9, 55.7 ± 7.8, and 61.3 ± 10.8 µmol/100 ml, respectively). Mucosal wet weight, protein, RNA, sucrase, and maltase of group 4 were significantly increased ($P<0.05$) compared with groups 2 and 3. Villus height was increased ($P<0.05$) in the jejunum of group 4 rats compared with groups 2 and 3, but not in any other segments of the intestine. No significant changes were observed in crypt depth among all groups. Diamine oxidase in groups 2, 3 and 4 was decreased ($P<0.05$) compared with group 1 in all segments except for the ileum. Significant decreases in mucosal weight, RNA content, maltase, sucrase, and diamine oxidase activity were observed in groups 2 and 3 compared with group 1. The results provide further evidence that administration of S-TPN solution results in significant mucosal atrophy. The results also indicate that the specific addition of Ala-Gln to S-TPN may preserve or enhance intestinal mucosal cellularity and function. Thus, alanyl-glutamine, the stable form of glutamine, may be a suitable form for inclusion in TPN formulas.

**Tarjan V, and G. Janossy. 1978.** The role of biogenic amines in foods. *Nahrung* 22 (3): 285-289.

3 Cheddar cheese samples made in Hungary averaged 13 mg tyramine/100 g (range 6.7-21.0) and 18 Emmental samples averaged 12.87 mg/100 g (5.8-36.0). These levels are considered dangerous for tyramine-sensitive consumers. Among 61 semi-hard cheese samples, tyramine content/100 g ranged from an average value of 1.32 mg for 5 Lajta samples to 3.6 mg for 4 Vadasz samples. For 21 processed and smoked cheese samples, average tyramine content was only 0.9 mg/100 g. Tyramine levels found in fruit, vegetables, fish, wines, chicken liver and sauerkraut are also tabulated (highest average level being in 4 kohlrabi samples, 93 mg/100 g).

**Tawfik, N. F., A. R. Shalaby, and B. A. Effat. 1992.** Biogenic amine contents of Ras cheese and incidence of their bacterial producers. *Egyptian Journal of Dairy Science* 20 (2): 219-225.

50 samples of Ras cheese from commercial sources in Cairo, Egypt, had mean amine-producing enterococcus counts of 1.6 X 105 (range 1.1 X 104 to 7.9 X 105) c.f.u./g, but no amine-producing lactobacilli were detected. Further examination of 20 samples for biogenic amines revealed the presence of tyramine in 8, histamine in 8, phenylethylamine in 9, putrescine in 16 and cadaverine in 18 samples; tryptamine was not found in any sample and 2 samples did not contain any of these amines. Detection limit

was 0.4 mg/100 g for tryptamine and phenylethylamine, and 0.2 mg/100 g for the other amines.

**Taylor, S. L. 1986.** Histamine food poisoning: toxicology and clinical aspects. *CRC Crit. Rev. Toxicol.* 17 (2): 91-128.

Histamine poisoning can result from ingestion of food containing unusually high levels of histamine. Fish present the most common source of poisoning. This article reviews various aspects of histamine food poisoning including: clinical aspects (definition, symptomology, diagnosis, treatment); epidemiology (world-wide, Japan, USA, UK); foods implicated in histamine poisoning (fish, cheese, chicken, sauerkraut); constraints to surveillance; formation of histamine and its control (low temp. storage and hygiene practices); analytical methods for detection of histamine; toxicology; and histamine metabolism.

**Taylor, S. L., L. S. Guthertz, M. Leatherwood, F. Tillman, and E. R. Lieber. 1978.** Histamine production by food-borne bacterial species. *Journal of Food Safety* 1 (3): 173-187.

A total of 112 bacterial strains representing 38 spp. were tested for their potential to elicit food poisoning outbreaks via histamine formation in foods. Proteus morganii and Enterobacter aerogenes displayed a quantitative superiority in terms of histamine production on a trypticase-soy broth- histidine (TSBH) medium and a tuna fish infusion broth (TFIB). When bacteria were incubated under standardized conditions in TSBH medium, histamine accumulated to levels exceeding 50 nmol/ml of media with a total of 23 strains, including 13 of 15 P. morganii strains, 3 of 3 E. aerogenes strains, 3 of 12 Hafnia alvei strains, 1 of 4 Providencia alcalifaciens strains, 1 of 5 Enterobacter cloacae strains, 1 of 1 Proteus rettgeri strains, and 1 of 1 Citrobacter diversus strains. However, only 8 of the 15 P. morganii strains and the 3 E. aerogenes strains were capable of generating histamine in excess of 200 nmol/ml in the TSBH medium. Of the 23 strains capable of appreciable histamine production in TSBH medium, P. morganii and E. aerogenes were, by far, the most prolific histamine producers in TFIB. Of the organisms tested, only P. morganii and E. aerogenes would appear to have the capability of forming sufficient histamine in scombroid fish products to elicit food poisoning outbreaks.

**Taylor, S. L., E. R. Lieber, and M. Leatherwood. 1978.** A simplified method for histamine analysis of foods. *Journal of Food Science* 43 (1): 247-250.

The method requires sample homogenization in methanol, heating, centrifuging or filtering, several extractions, and fluorometric detection of histamine with o-phthalaldehyde. It eliminates potential interference by other amines through a selective extraction step. Samples of 20 foods, including seafood products (fresh, frozen and canned), comminuted meats, cheeses and sauerkraut (canned), were analysed for histamine content. Canned sauerkraut and tuna fish had the highest average histamine content among tested foods. The method can be used to detect histamine in food samples that contain as little as 0.02 mg histamine/100 g.

**Teodorovic V, Buncic S, and D. Smiljanic. 1994.** [A study of factors influencing histamine production in meat.] Studie zu Faktoren, die die Histaminbildung im Fleisch beeinflussen. *Fleischwirtschaft ;* 74 (2): 181-183.

Histamine formation in nutrient broth (containing added histidine at 0.05 mol/l) inoculated with Proteus morganii (isolated from raw meat) was studied. Histamine formation was increased in the presence of 0.30% glucono-delta-lactone and 1.00% sucrose. Histamine formation in the presence of 3.50% NaCl or 0.02% NaNO2 was similar to that in control broth without additives. Ground meat and mackerel samples were inoculated with P. morganii (3 x 10-4 cells/g) and stored at 5-7 or 18-22 degree C until spoiled. Max. concn. of histamine formed at 5-7 and at 18- 22 degree C respectively were (mug/g): beef 10.58 and 33.67;

pork 22.49 and 36.03; mutton 18.14 and 30.89; poultry 61.73 and 77.18; and mackerel 981.30 and 1565.65. The relation between bacterial count and amount of histamine formed was studied in mackerel inoculated with P. morganii and stored at 5-7 degree C for 9 days. A substantial increase in histamine concn. was observed during the period when the bacterial ccunt remained approx. constant. [From En summ.] [An En version of this paper is published on pp. 170-172 of this issue of Fleischwirtschaft.].

**Torrigiani, P., Scoccianti, V. and Bagni, N. 1988.** Polyamine oxidase activity and polyamine content in maize during seed germination. *Physiologia Plantarum* 74: 427-432.

Polyamine oxidase (PAO) activity and polyamine content was investigated in the cell wall and soluble fractions obtained from embryos, endosperms and shoots and roots of etiolated or green seedlings of maize cv. WF9 during the 1st 7 d of germination. Polyamine content was also determined in the trichloroacetic acid (TCA) soluble (free polyamines) and TCA insoluble (bound polyamines) fraction obtained from the same tissues. PAO activity, determined by the radiometric method based on the recovery of the labelled reaction product 1-pyrroline, was mostly localized in the cell wall fraction. The activity was very low in embryos and endosperms and present in traces in roots. In etiolated shoots PAO activity increased sharply, while in green shoots it was low and increased slowly. No polyamines were found in the cell wall fraction and only putrescine was detected in the soluble fraction, with the exception of the embryo, where spermidine and spermine were also present. In the TCA-soluble fraction of embryos, putrescine increased during imbibition, while spermidine and spermine decreased; in the endosperm no relevant changes in polyamines occurred. In the same fraction of green and etiolated seedlings, putrescine increased, giving a peak at days 3-5, while spermidine decreased to very low levels. The amount of bound polyamines was 1-4% of the free ones. The pattern of PAO activity seemed to be unrelated to endogenous free polyamine content, which was the same in shoots and roots of etiolated and green seedlings. Enzyme activity, which was very low in ungerminated seeds, increased continuously during germination, especially in eliolated shoots, indicating a possible invovlement in cell wall formation.

**Treptow, H., and A. Askar. 1990.** Analytical methods for the estimation of biogenic amines in foods. *Emahrung* 14 (1): 9-11, 14-17.

The most suitable method for rapid, accurate and sensitive estimation of biogenic amines in a variety of foods, which is by column chromatography in an amino acid analyser or by high- performance liquid chromatography, using a range of extraction and cleaning techniques, is detailed in this review. Where such equipment is not available, thin-layer chromatography and gas chromatography can be used. For estimating histamine in food, fluorimetric estimation has yielded best results.

**Tsai, H., and S. G. Weber. 1990.** Electrochemical detection of dipeptides and dipeptide amides. *J. Chromatography 515: 451-457.*

A postcolumn reagent was used to create electroactive species from non-electroactive peptides. The reagent, based on the classical biuret reagent, consisted of Cu(II), tartrate, bicarbonate and base. Detection was by dual electrode electrochemical detection. N-Acetylated dipeptides were oxidized at pH 12 and low potential. Dipeptides and carboxy terminal dipeptide amides gave useful signals at lower pH. Dipeptide amides reacted with Cu(II) as do tripeptides to yield the electronic spectrum and electrochemistry of the biuret complex. The complexes formed from the dipeptides were reversibly oxidized at potentials greater than 0.85 V vs. Ag/AgCl, 3 M NaCl. Analytically useful signals were obtained for the dipeptide amides at sensitivities equivalent to the sensitivities for longer peptides, 3 to 5 nA/μM, whereas the sensitivities for the dipeptides were about an order of magnitude lower.

**Tschabrun, R., K. Sick, F. Bauer, and P. Kranner. 1990.** Histamine production in firm dry sausages. *Fleischwirtschaft* 70 (4): 448-552.

The histamine content in firm dry sausages, its production during ripening and the influence of technological variables on the level of the histamine content were examined. The microbiological status was also considered and the ability of microorganisms to produce histamine tested. In Austrian dry sausages, irrespective of variety, histamine content varied between 1 and >600 mg/kg DM. Most of the histamine was produced during the first two to four weeks of ripening. Ripening conditions had no influence on the level of the histamine contents. A small decrease could be achieved by using nitrite in products normally cured with nitrate. Histamine content could be decreased by using particularly fresh meat. Total aerobic count and concentrations of lactobacteria were about twice as high in dry sausages with a high histamine content. Presence of histamine- producing organisms and favourable conditions for microorganisms caused excessive histamine production.

**Tucker, D. J., G. P. Jones, and D. E. Rivett. 1983.** Formation of beta-phenylethylaminoalanine in protein foods heated in the presence of added amine. *J. Science of Food and Agriculture* 34 (12): 1427-1433.

Reaction of phenylethylamine with a variety of protein- containing foods was demonstrated. The reaction resulted in formation of a beta-N- substituted diaminopropionic-acid derivative, D l-3-(N-phenylethylamino)-alanine (PEAA), presumably by a pathway analogous to the formation of lysinoalanine. PEAA was detected in 3 cheeses, 2 soy products and a concentrated yeast extract after heating with added amine at various pH values. It was also found in a cheese which had been subjected to a simulated grilling procedure. Of considerable interest was the detection of this novel amino acid in foods heated with amine below pH 7. It was earlier proposed that PEAA may be produced by a mechanism similar to that attributed to formation of lysinoalanine which is often found in alkali-treated foods. This type of reaction, however, would not be expected to occur at acidic pH and the production of PEAA may involve an alternative mechanism. Experiments with tyramine and histamine indicated that this reaction may occur with a variety of biogenic amines.

**Umemura, Y., Miyazaki, S., Yamanaka, H., Ohya, T., Homma, S., Oka, M., Sato, S. and Nakahara T. 19??.** Properties of gizzard erosion inducing substance in fish meal.xxxxxxxx yy: zzz-zzz.

Heated whole fish meal produced from mackerel induced gizzard erosion in broiler chicks after feeding for 6 days. Constituents of the water-soluble fraction of fish meal were suspected to cause this lesion. His, histamine and nucleotides were examined. When added to milk casein and heated, His appeared to be the most toxic. Histamine and IMP might be less toxic even when treated in the same way as His. Feeding of a large amount of histamine alone resulted in disturbed profile of body weight gain and a low incidence of gizzard erosin. Heated fish meal and a heated mixture of casein and His were easily reduced in toxicity by acid hydrolysis. The latter was more sensitive to this treatment. Papain did not diminish the toxicity of heated fish meal. Even after treatment with papain, the heated casein-His mixture was still effective to induce gizzard erosion. Results might have come from different structural changes caused by heating of the 2 samples.

**Urlings, H. A. P., P. G. H. Bijker, and J. G. v. Logtestijn. 1993.** Fermentation of raw poultry byproducts for animal nutrition. *J. Anim. Sci. 71 (9): 2420-2426.*

In this study, the fermentation of raw, inedible poultry byproducts mixed with sugarbeet pulp and dextrose and inoculated with Lactobacillus plantarum and(or) Enterococcus faecium resulted in a drop of pH in the byproducts to approximately 4.0 to 4.5 within 48 h. To keep the fermented product stable for a period of 21 d, the addition of greater than or equal to 3% (wt/ wt) of a fermentable carbohydrate was necessary. With a high inoculation level of approximately 10(8) to 10(9) L. plantarum per gram, or with acidification of the

initial mixture with .4% lactic acid, the number of Enterobacteriaceae decreased faster than with inoculation at 10(6) L. plantarum per gram, or without initial acidification. After 21 d of fermentation, a high level of enzymatic breakdown of proteins and amino acids was observed: the nonprotein N level increased from 5% to between 15 and 40% of total N and the volatile N level increased from 1% to between 3 and 11% of total N. An increase in histamine, cadaverine, and putrescine was also observed. Despite the technological measures taken, such as the application of a high inoculum of starter culture and initial acidification with .4% lactic acid, this amino acid breakdown could not be reduced to an acceptable level. These results suggest that, because of biochemical deterioration, fermentation alone is not a useful method of preservation of raw poultry byproducts.

**Urlings, H. A. P., N. G. Fransen, P. G. H. Bijker, and J. G. v. Logtestijn. 1993.** Proteolysis and amino acid breakdown of heated and irradiated poultry byproducts and muscle tissue. *J. Anim. Sci. 71 (9): 2432-2438.*

As a result of intensification and centralization of poultry slaughtering, the amount of slaughter byproducts produced at a single location is increasing. These byproducts are rich in protein, fat, and vitamins and, therefore, constitute a potentially useful raw material for use as animal feed. To maintain the nutritive value of these byproducts they should be processed to minimize or eliminate degenerative changes that reduce the feed value of the product. In this paper amino acid breakdown in slaughter-fresh poultry viscera, heads, and breast meat is studied as a model. Initial amino acid breakdown in viscera was observed (also when bacterial growth was excluded by gamma-irradiation), which resulted in high levels of total volatile N and cadaverine. Putrescine was produced only in viscera after bacterial proliferation. In heads and breast meat, no production of metabolites of amino acid degradation was observed as a result of initial enzymatic activity. It is concluded that during preservation of poultry byproducts not only bacterial proliferation, but also enzymatic breakdown of amino acids, must be prevented.

**Vidal Carou, M. C., M. L. Izquierdo Pulido, M. C. Martin Morro, and A. Marine Font. 1990.** Histamine and tyramine in meat products: relationship with meat spoilage. *Food Chemistry* 37 (4): 239-249.

Biogenic amines in processed meat products can be useful as indices of poor-quality raw material, but they can also be related to microbial activity involved in fermentation processes. New data on this topic are presented. Histamine, tyramine and pH changes were followed during storage/spoilage of beef and pork at room and refrigerated temperatures. A notable increase in the content of amines was observed at both temperatures studied. There was greater and more rapid formation of histamine and tyramine in pork than in beef. The increase in the amines occurred before the increase in pH value. A preliminary study on the influence of ripening on histamine and tyramine contents was also made. This process seems to exert a greater influence on histamine than on tyramine content. Finally, the histamine and tyramine contents of 63 Spanish meat products were estimated. Both amines were detected in all samples, but concentrations varied greatly. Uncooked and ripened meats showed significantly higher values for both amines than did cooked meat products. In cooked meat products, histamine values ranged from 0.25 to 3.90 and tyramine from 0.50 to 25.6 mg/kg. For uncooked and ripened meat products, the range of histamine was from 0.25 to 249 and of tyramine from 0.45 to 510 mg/kg.

**Voigt, M. N., and R. R. Eitenmiller. 1977.** An evaluation of extraction and thin layer chromatographic procedures for the quantification of biogenic amines in foods. *Lebensmittel Wissenschaft und Technologie* 10 (5): 263-267.

Of 9 extraction procedures evaluated for their abilities to give max. extraction of tyramine, histamine and tryptamine added to cheese, the alkaline-polar method of Lovenberg & Engelman [in 'Methods of biochemical analysis', Glick (editor), Interscience Publishers (1971)] was best since it was selective for the 3 amines. The ion-exchange procedure of Blackwell & Mabbitt [Lancet (1965) 1 (7392) 938] was not as selective for the 3 amines but was useful because it extracted other basic compounds. Of 22 TLC solvent systems tested the best were chloroform/methanol/NH4OH (12:7:1), which provided the best resolution, and n-butanol/ pyridine/acetic acid/water (15:2:3:5) which completely separated tyrosine, tyramine, tryptophan, tryptamine, histidine and histamine. The 2 extraction procedures and the TLC solvent systems

have been routinely used in studying the biologically active amine content of foods.

**Voigt, M. N., and R. R. Eitenmiller. 1974.** Fluorescent quantitation of biologically active amines in foods with 7-chloro-4- nitrobenzofurazan (NBD-Cl). *J. Food Sci.* 39 (2): 420- 421.

The usefulness of NBD-Cl for the fluorescence detection of biologically active amines commonly found in fermented foods was studied. Limits of detection of down to 0.1 mug/TLC spot are given for 11 amines added to Cheddar cheese. NBD-Cl was found to be superior to o-phthaldehyde and ethylene diamine due to the simplicity of derivative formation and ability to form fluorescent derivatives with 10 of the 11 amines. NBD-Cl was shown to form quantitative derivatives with tyramine, tryptamine and histamine. There was a limited linear response for tryptamine.

**Vuorela, H., R. Hinkkanen, and R. Hiltunen. 1989.** Rapid determination of tyramine in fish feed and slaughter offal by HPLC using coulometric detection. *Zeitschrift fur Lebensmittel Untersuchung und Forschung* 189 (5): 434-437.

Fish feed and slaughter offal products may contain decomposition compounds such as biogenic amines. Owing to their harmful effects on animals fed with such products, there is a need for estimating the amine content. A simple and fast HPLC method, based on coulometric detection (EC) measuring only tyramine, was developed for routine quality screening. The samples were extracted with 0.4 mol/litre perchloric acid and analysed directly by HPLC using a mobile phase of 0.1 mol/litre potassium dihydrogen phosphate in water at pH 3. Tyramine was detected using a coulometric detector consisting of 2 analytical cells, the first one at 0.4 V and the second at 0.7 V. Calibration was linear over the range 4.52 to 452 ng/ml and minimum detectable quantity was 10 pg/20 µl. Reproducibility and recovery was high. Comparison of the tyramine content measured by EC or derivatization followed by ultraviolet detection showed that both methods gave similar results. HPLC using EC is a fast and sensitive method for analysing tyramine reliably in fish feed and slaughter offal samples without any time-consuming derivation steps.

**Wang, L.C. 1973.** Polyamines in soybeans. *Plant Physiol.* 50: 152-156.

Polyamines were extracted from soybean flour with trichloroacetic acid and separated by cationic exchange column chromatography. Putrescine, spermadine and spermine were identified by TLC, paper electrophoresis, mass spectral analysis, reactions with ninhydrin and Dragondorff reagents, and spectrophotometric characteristics. Soybean flour contained 28.7 mug polyamines/g (estimated by N analysis on the pooled fractions). Resting seeds contained higher concn. of spermidine than of spermine or putrescine; the concn. of spermine was lower than that of putrescine. The putrescine content increased rapidly on germination. Polyamines were also present in the alcohol-soluble fraction; some soybean polyamines appear to be present in bound forms. Polyamines may cause off-flavours in soy flour, limiting its utilization in foods; alcohol extraction appears to be an effective method for removal of polyamines from soybeans.

**Wang, L.C. and Selke, E. 1973.** Soybean polyamides. Separation and characterization of cadaverine. *Plant Physiol.* 51: 432-435.

**Wang, L. C., Cavins JF, and Wolf WJ. 1978.** Gamma-glutamyl dipeptide content in soybean protein products and other commodities. *Journal of Food Science* 43 (3): 740-742.

The amount of gamma-glutamyl dipeptides (gamma-glutamyl tyrosine and gamma-glutamyl phenylalanine)

present in soybeans and other commodities was measured by a method consisting of alcohol extraction, preliminary purification and detn. with an automated amino acid analyzer. Defatted soybean flakes of 4 var. and 1 commercial sample contained 1.17-1.60 mg of the dipeptides/g. Most of the dipeptides in soybean concentrates and isolates were lost through processing (contents 0.02-0.1 mg/g). Corn, wheat, cottonseed meal, pork, beef, chicken and lamb appeared to contain only traces, but peanut flour had a content of 0.20 mg/g. The method adequately measures 50 mug of the dipeptides and recovers greater than 95% of gamma-glutamyl phenylalanine standard added to samples of soybean flour and meat-soybean flour mixtures.

**Watts, D. A., and W. D. Brown. 1982.** Histamine formation in abusively stored Pacific mackerel: effect of CO2-modified atmosphere. *Journal of Food Science* 47 (4): 1386-1387.

Whole Pacific mackerels (Scomber japonicus) were abusively stored at 20 degree C in air or 80% CO2, balance air. Samples were analysed for amines using a modified amino acid analyzer. Following 24 h storage, levels of histamine, tyramine, putrescine, and cadaverine increased only slightly above the low levels observed initially. During the next 24 h, the amine content increased dramatically. Levels in the air control samples were about twice those in the modified atm samples. In a separate trial, amine levels in fish stored 3 days were higher still and similiar in the 2 atm. Thus, in neither trial did CO2-modified atm storage lead to increased production of potentially toxic amines.

**Wendakoon, C. H., and M. Sakaguchi. 1993.** Combined effect of sodium chloride and clove on growth and biogenic amine formation of Enterobacter aerogenes in mackerel muscle extract. *Journal of Food Protection* 56 (5): 410-413.

Inhibitory effects of clove (0.5%) and NaCl (1-5%) on growth and biogenic amine (histamine and cadaverine) production by Enterobacter aerogenes [ATCC 43175] in mackerel muscle broth at 30 degree C were investigated. At the 1% level, NaCl was favourable for growth; higher levels slightly reduced growth. Max. population numbers obtained in the presence of NaCl were the same as controls. Amine production was enhanced by the presence of 1% NaCl alone. Only a small increase was observed at higher levels; NaCl at greater than 3% had no stimulatory effect on amine formation. Addition of clove (0.5%) to the broth resulted in delays in growth and amine formation. NaCl at concn. as low as 2% in combination with clove (0.5%) completely inhibited growth and amine production of E. aerogenes in mackerel broth. It is suggested that synergistic effects of clove essential oils and NaCl could be considered as the probable reason for inactivation of E. aerogenes. [From En summ.].

**Windyga, B., A. Grochowska, H. Sciezynska, K. Gorecka, and M. Fonberg Broczek. 1992.** Estimation of histamine in fish preserves by use of the colorimetric procedure by Hardy-Smith. *Roczniki Panstwowego Zakladu Higieny* 43 (2): 193-199.

Oznaczanie histaminy w konserwach rybnych metoda kolorymetryczna wg Hardy-Smitha.

The histamine content of 79 samples of canned fish was estimated using the method by Hardy-Smith. Of 45 samples of canned sardines imported from Yugoslavia 6, 2, 6, 9 and 22 samples contained histamine above 50, from 30.1 to 50, 20.1 to 30, 10.1 to 20, and up to 10.0 mg/100 g, respectively. Of 29 samples of canned sardines imported from the Soviet Union 11 and 18 samples had histamine from 10.1 to 20.0 and up to 10.0 mg/100 g, respectively. The content of histamine in 5 samples of canned mackerel imported from the Soviet Union did not exceed 10.0 mg/ 100 g. The procedure used was recommended for routine estimations of histamine in canned fish.

**Yamamoto, S., H. Itano, H. Kataoka, and M. Makita. 1982.** Gas-liquid chromatographic method for analysis of di- and polyamines in foods. *Journal of Agricultural and Food Chemistry* 30 (3): 435-439.

A GLC method for quantitative detn. of putrescine, cadaverine, spermidine, and spermine in foods has been developed. The amines were separated from 31 different types of food by eluting through a cation-exchange resin column and then converted to their (ethyloxy)carbonyl derivatives by reaction with ethyl chloroformate in aqueous medium before application to the gas chromatograph with a flame ionization detector. 1,8- Diaminooctane was used as internal standard. Separation and determination of the resulting derivatives were performed on a 1.5% SE-30/0.3% SP-1000 on Uniport HP column (0.5 m) under the temperature-programmed condition. The calibration curves for the amines in the range of 12.5- 125 nmol were linear and sufficiently reproducible for quantitative detn. Overall recovery rates were satisfactory. Putrescine and spermidine were present in all the foods investigated. Relatively large amounts of spermidine occurred in mushrooms and beans (up to 52.24 and 52.74 mg/100 g, resp.).

**Yamamoto, S., S. Wakabayashi, and M. Makita. 1980.** Gas- liquid chromatographic determination of tyramine in fermented food products. *Journal of Agricultural and Food Chemistry* 28 (4): 790-793.

Tyramine is extracted from foods with 2% $HClO_4$, purified by Amberlite CG-120 chromatography, and converted into the N,O-bis (ethyloxycarbonyl) derivative by reaction with ethyl chloroformate of room temp. This derivative is stable enough for quantitative GLC detn. by using a 1.5% OV-17, 0.2% SP-1000 mixed phase column. 3,4-dimethoxyphenethylamine is used as an internal standard. Tyramine is well separated from other amines under the conditions used. Derivatization yields were almost quantitative, and the calibration curve was linear for 0.04-2.0 mug tyramine injected. Coeff. of variation of 5 repeated assays on foods such as soy sauce, sake, soybean paste, fermented milk, beer and cheese were less than 6.2, and recoveries from these foods fortified with tyramine were 94.1-98.0%.Commercial samples of the same foods were analysed for tyramine; ranges of contents found, resp. (mug/ml or /g) were 136.6-882.0, 0.21-0.51, 0.21-169.5, 0.41-2.33, 1.13-1.30, and 29.8-138.4. As well as tyramine, detn. of other amines such as putrescine, cadaverine, beta- phenethylamine and N-methyltyramine may be possible.

**Yang, P., Baylin, S.B. and Luk, G.D. 1984.** Polyamines and intestinal growth: absolute requirement for ODC activity in adaptation during lactation. *Am J Physiol.* 247: G553-G557.

Ornithine decarboxylase (ODC), through the regulation of polyamine biosynthesis, is important in cell proliferation and differentiation. Intestinal mucosal ODC activity was studied in lactating Lewis rats and the ODC value was correlated with the characteristic small intestinal adaptive changes accompanying lactation. During the first 14 days of lactation, mucosal ODC activity increased, with the maximum increase on day 5 corresponding to the time of maximum morphological intestinal adaptation. In rats given the specific inhibitor of ODC, alpha- difluoromethylornithine (DFMO), intestinal mucosal ODC activity was inhibited, and intestinal adaptation was suppressed, with diminution of the adaptive increase in mucosal weight and thickness especially in crypt depth. The results suggest that ODC activity plays an essential role in mucosal hyperplasia during intestinal adaptation accompanying lactation, possibly through stimulation of crypt cell proliferation.

**Zee, J. A., Simard RE, and L. L'Heureux. 1983.** Evaluation of analytical methods for determination of biogenic amines in fresh and processed meat. *Journal of Food Protection* 46 (12): 1044-1049.

15 biogenic amines were separated and quantitated by an automated ion-exchange chromatography technique. Extraction efficiencies for amines from fresh and processed meat [round steak, pastrami, pork belly, bacon, pork butt, ham, chicken emulsion and wiener] using trichloroacetic acid (TCA), perchloric acid and methanol were compared. In general, biogenic amines in meat and meat products were better extracted by TCA. Aliphatic amines were more efficiently extracted than aromatic amines. Type of meat

and adsorption of amines on proteins probably affected extraction efficiency. Both fresh and processed meat products contained high amounts of adrenaline, spermidine and spermine (up to 581, 280 and 685 mg/kg, resp.), but low amounts (13-19 mg/kg) of noradrenaline, putrescine, histamine, cadaverine and tyramine. Processed meat contained lower concn. of amines than fresh meat, suggesting losses during salting and curing or microbial growth inhibition.

# 2. Industry Survey

## Sample Preparation and Procedure

Samples were minced and representative samples were taken for the analysis of the three biogenic amines, putrescine, cadaverine and histamine which were extracted with 0.1N HCl. The amines were derivatised with dansyl chloride and purified using a liquid - liquid extraction. Biogenic amines were determined in all samples by High Performance Liquid Chromatography (HPLC) using a C18 reversed phase column and a UV detector.

## Questionnaire Response

Companies completed a questionnaire (Refer Appendix 1) which provided a basic history of the sample supplied. Results of the survey indicated that five rendering processors, namely dry batch rendering, continuous dry rendering, digestor wet rendering, low temperature rendering, semi continuous dry rendering and high temperature rendering are used in Australia. The first two being the most widely used. Time delays indicate the time that passed from the kill to commencement of cooking. These delays were mainly due to transportation and occasional equipment breakdown within the rendering plant. Prior to rendering, the majority of raw material was held at ambient temperature at the rendering site or during transportation to the rendering site. Temperatures ranged from 17°C to 40°C. Digestive tract organs were present in the majority of raw material. Composition of raw material ranged from 100% beef to varying compositions of beef, mutton, pork, fat and bone. Material from knackeries was present in only a few samples. The particle size in most of the raw material was reduced prior to rendering. Reducing the particle size of the raw material may release the bacterial amino decarboxylase enzymes required fro the conversion of amino acids to biogenic amines.

Other informative responses to the questionnaire completed by the rendering companies were obtained. The renderers indicated that they are aware of time delays existing between the kill and the commencement of rendering. The majority of renderers perform routine maintenance on their equipment and record temperatures throughout the cooking process. Routine testing of random meatmeal samples for determination of protein, fat, moisture and ash were carried out by 80% of renderers. This routine testing however varied from weekly to quarterly at rendering sites. The remaining 20% of rendering companies were able to provide levels of minimum fat and protein levels of their meatmeals; however they did indicate that no control measures were undertaken to ensure the specifications were met. In one case, a company did not know which rendering process was employed.

## Dry Batch Rendering

The raw material in dry batch rendering is loaded through a top door into the cooker and is indirectly heated to approximately 130°C while being mechanically agitated. The internal pressure of the cooker can be increased or decreased as required through the adjustment of a vent. The raw material is cooked for approximately 2-3 hours. Once cooking is complete, the cooked material is loaded onto a pan where the fat is drained

and removed. The solid material is then pressed to remove the remaining fat. The material is then milled. Dry batch rendering has the advantages of being able to cook, pressurize and sterilize in the one vessel. Minimal loss of material occurs from the cooker and hot water can be provided from the vent steam of the cooker. The disadvantages range from a dark tallow being produced, the meal fat content of 10-16% is high compared to other processes, the raw material needs to be cut and washed therefore adding water to the sample and resulting in loss of protein and fat. The process is labour intensive.

Forty seven samples submitted were processed by dry batch rendering (Table 2.1, and Graphs 2.1 and 2.2). The majority of the samples were rendered within 6 hours after the kill. In one case, up to 36 hours passed before the raw material was processed. The raw material consisted of varying proportions of beef, mutton, pork, fat, bone and offal. In the majority of raw material, the digestive tract organs were present and the particle size was reduced prior to rendering. No significant differences were observed in the total levels of amines for those samples whose digestive tract organs were or were not included and particle size reduced or not reduced. The raw material was kept at ambient temperature. Cooking times of the raw material ranged from 1.5 to 3 hours. Cooking temperatures ranged from 100-160°C. Rendering systems with no pressure had cooking temperatures as high as 550°C. Levels of putrescine, cadaverine and histamine ranged from 2-129 mg/kg, 3-324 mg/kg and 5-41 mg/kg, respectively. Total levels of the three amines ranged from 20-494 mg/kg. The results of the total levels of amines present in the samples analysed showed that 76% < 100 mg/kg, 15% = 100-200 mg/kg and 9% > 200 mg/kg.

Eleven samples with time delays between 5 to 36 hours had total levels of amines greater than 100 mg/kg. Thirty six samples with time delays between zero to 8 hours had total amine levels less than 100 mg/kg. These results indicate that the time delay between the kill and cooking played a crucial role in the production of biogenic amines.

Samples 10A and 10B were rendered by the same company. Sample 10A was approximately 2.5 hours old when rendered and comprised of 100% beef. The particle size was reduced progressively over 1 hour prior to rendering. Sample 10B was approximately 11 hours old when rendered and comprised of 100% beef. The particle size was reduced 11 hours prior to rendering. Both samples were cooked at 100°C at 97 psi. Sample 10A was cooked for 1 hour 55 minutes and sample 10B for 2 hours 20 minutes. Sample 10A had a total amine level of 66 mg/kg and sample 10B had a total amine level of 494 mg/kg. These results indicate the time delay has had a significant impact on the total level of biogenic amines produced.

Samples 20A, 20B and 20C were supplied by the same company. The three samples contained 100% beef and had time delays of 0.5, 2 and 8 hours respectively. The digestive tract organs were present and the particle size of the raw material was reduced in each sample. The total amine levels of 44, 38 and 48 mg/kg indicate that the levels did not increase with increasing time delays. Samples were cooked at 105°C for 120-150 minutes. Sample 20C was held at 80°C overnight for 8 hours. Holding sample 20C at this temperature has prevented the bacteria from functioning and it was therefore unable to convert the amino acids to their corresponding biogenic amines.

Sample 18 was red meat offal and had a total level of 214 mg/kg putrescine, cadaverine and histamine. This raw material had a time delay of 10 hours. The time delay together with the bacteria present in the offal provide ideal conditions for the bacteria to produce the high levels of total amines.

Samples 41A to 41V were submitted by the same company. The samples ranged from 100% beef to varying compositions of beef, bone, fat, pork and mutton. The highest total level of amines found in any of these samples was 73mg/kg. The digestive tract organs were present in the raw material and the particle size was reduced prior to rendering. The raw material was rendered immediately after the kill, therefore reducing the time for the bacterial enzymes to deaminate the amino acids.

**Continuous Dry Rendering**

The raw material in continuous dry rendering is treated in a similar manner to that of dry batch rendering. The main difference is that the machinery is designed for continuous loading and discharging of the meatmeal. The advantages of continuous dry rendering are that less floor space is required and it is not as labour intensive as dry batch rendering. The vent steam can be used to provide hot water and there is minimal loss of material. The disadvantages are that the system cannot sterilize by pressure and therefore is unable to hydrolyse hair and wool. The system has similar disadvantages to those of dry batch rendering.

Twenty seven samples submitted were processed by continuous dry rendering (Table 2.2, Graphs 2.3 and 2.4). The majority of the samples were processed within 4 hours. Some raw materials were 24 hours old. The raw material contained varying proportions of beef, mutton and pork. The digestive tract organs were not included in the raw material of samples 12 and 14. The raw material was kept at ambient temperature. Cooking times of the raw material ranged from 20 minutes to 3 hours. Cooking temperatures of the raw material ranged from 120°C-150°C. The levels of putrescine, cadaverine and histamine varied from 7-293 mg/kg, 6-329 mg/kg and 7-34 mg/kg, respectively. Total levels of the three amines ranged from 34-656 mg/kg. The results of the total levels of amines present in the samples analysed showed that 37% < 100 mg/kg, 41% = 100-200 mg/kg and 22% > 200 mg/kg.

Samples 17A to 17L were submitted by the same company. Samples 17A to 17D had high total levels of amines ranging from 250-656 mg/kg. The raw material of these four samples were approximately 24 hours old when rendered. Samples 17E to 17G had total amine levels ranging from 124-162 mg/kg and had time delays of approximately 3 hours. Samples 17H and 17I were approximately six hours when rendered and had total levels of 170 and 247 mg/kg, respectively. Samples 17J to 17L were a combination of the cooked samples 17A to 17 I. The large time delay for samples 17A to 17D influenced the levels of amines produced.

Sample 24 was 4-6 hours when rendered and the raw material was kept between 30°C to 40°C. The raw material consisted of 100% beef. The digestive tract organs were present and the particle size of the raw material was reduced. The time delay and high temperatures did not result in high levels of amines.

The raw material of sample 28 consisted of soft offal held at 32°C for 4 hours and boning material held at 10°C up to 36 hours. Despite the time delay, the total amine level of 83 mg/kg was quite low. This could be due to the proportion of offal to boning material when rendered.

## Digestor Wet Rendering

The raw material in digestor wet rendering is cooked using a direct injection of steam at a temperature of approximately 140°C and a pressure of 360 kPa. Loading of the raw material may take up to 8 hours. During loading, the raw material is kept gently boiling to prevent degradation of the fat by bacterial enzymic action. The heat transfer medium in this type of rendering is water and therefore depending on the moisture content of the raw material it may be necessary to add water. When the digestor is fully loaded, steam is injected into the raw materials at a temperature of 140°C and at a pressure of 360 kPa. Cooking proceeds from 2-6 hours during which time fat floats to the top of a watery layer and the solid materials settle on the bottom of the digestor. After cooking is completed, the fat and watery layer are removed by using high internal pressure. The solid material is transferred to driers to remove the water by pressing, centrifuging or heat. The material is then milled. The advantages of digestor wet rendering are that a good quality tallow can be produced provided that the viscera is washed thoroughly. The disadvantages are that the operation is very labour intensive, the system has long cook times and up to 25% of the meal can be lost in the watery layer.

Three samples submitted were processed by digestor wet rendering (Table 2.3; Graph 2.5). The digestive tract organs were not present in the raw material of sample 11. The particle size of the raw material was reduced in sample 35. The raw material of sample 11 and 35 were kept at ambient temperature. Cooking times of the raw material ranged from 1.5 to 2.5 hours. Viscera of sample 23 was cooked for 4-5 hours. Cooking temperatures were approximately 125°C and pressure near 650 kPa.

The levels of putrescine, cadaverine and histamine varied from 18-42 mg/kg, 17-56 mg/kg and 16-43 mg/kg respectively. Total amine levels of the three samples ranged from 51-127 mg/kg. Samples 11 and 35 had time delays up to 2 hours while the raw material of sample 23 had viscera material up to 20 hours old and butchers' material up to 7 days old when rendered. The raw material of sample 23 was held at a temperature of 5°C. At this temperature, the activity of the decarboxylase enzymes to convert amino acids to biogenic amines was reduced significantly and therefore the extensive time delay did not result in high levels of amines.

## Low Temperature Rendering

Low temperature rendering systems operate at approximately 95°C and can process 10 tons or more raw material per hour.

Pre-broken raw material passes through a metal detector and then into a heavy duty grinder where the particle size is reduced to approximately 12 mm. The ground material is transferred into the cooker at a controlled flow. High speed agitators ensure high rates

of heat transfer and a low degree of fat emulsification. Once the fat is separated, the solid material enters a drier in which the temperature of the meal is increased to approximately 110°C for 20-30 minutes. The material is then milled. The advantage of low temperature rendering is that the low cooking temperature reduces power costs for the rendering plant.

Four samples submitted were processed by low temperature rendering (Table 2.4; Graph 2.6). The digestive tract organs were present in all four samples. Cooking temperatures ranged from 80°C to 130°C. Cooking times ranged from 5-20 minutes. Drying times of the samples ranged from 20 minutes to 3.5 hours depending on the quantity and type of raw material. The levels of putrescine, cadaverine and histamine ranged from 9-350 mg/kg, 6-430 mg/kg and 13-58 mg/kg respectively. Total levels of the three amines ranged from 28-832 mg/kg. Time delays of the raw material of samples 2, 9 and 21 were no greater than 6 hours. Sample 42 had a time delay of up to 36 hours and a high total amine level of 832 mg/kg. This long time delay allowed sufficient time for the bacterial enzymes to convert the amino acids to biogenic amines.

## Other Rendering Systems

Sample 33 was submitted from a semi-continuous rendering system with a time delay of 5 hours. Putrescine, cadaverine and histamine levels were 96, 182 and 20 mg/kg, respectively. Sample 42B was submitted from a high temperature rendering system with a time delay of up to 36 hours. Putrescine, cadaverine and histamine levels were 155, 163 and 23 mg/kg, respectively.

Three samples that were supplied had no information about the type of rendering process. These three samples had total amine levels of 191, 45 and 27 mg/kg.

Samples 5A and 5B were supplied by the same company. Sample 5A was produced using dry batch rendering and sample 5B using continuous dry rendering. Both samples were rendered from the same material which consisted of 65% beef, 30% mutton and 5% pork. Both samples had time delays up to 5 hours. Sample 5A was cooked for 40 minutes at 160°C and sample 5B was cooked at 120°C-135°C for 40 minutes. The total amine levels of 5A and 5B were 161 and 229 mg/kg, respectively. These results indicate that the cooking temperature and cooking time have contributed to the different levels of biogenic amines present in the meals.

Samples 42A and 42B were supplied by the same company. Sample 42A was produced using low temperature rendering and sample 42B was produced using high temperature rendering. Both samples were rendered from the same raw material and had time delays of up to 36 hours. Sample 42A was preheated at 94°C at 1 metre/3 minutes and dried at 110°C at 1 metre/20 minutes. Sample 42B was cooked at 132°C at 1 metre/ 12 minutes. The total amine levels of 42A and 42B were 832 and 341 mg/kg, respectively. These results indicate that the temperature of the cooker, the rendering process and the time delay contribute to the varying biogenic amine levels in meatmeals.

## Summary

Table 2.5 is a summary of the total levels of putrescine, cadaverine and histamine contained in Australian meatmeals from the four main rendering processes, dry batch rendering (DBR), continuous dry rendering (CDR), digestor wet rendering (DWR) and low temperature rendering (LTR). The total amine levels have been placed in three main categories of $<100$ mg/kg, 100-200 mg/kg and $>200$ mg/kg. The cooking temperatures and times have been included.

Table 2.5

| Rendering Process | Number of Samples Analysed | <100mg/kg % | 100-200mg/kg % | >200mg/kg % | Cooking Temperature $^0C$ | Cooking Time Hours |
|---|---|---|---|---|---|---|
| DBR | 47 | 76 | 15 | 9 | 100-160 | 1.5-3 |
| CDR | 27 | 37 | 41 | 22 | 120-150 | 0.3-3 |
| DWR | 3 | 67 | 33 | - | 125 | 1.5-2.5 |
| LTR | 4 | 75 | - | 25 | 80-130 | $_{cooking}$ 0.1-0.3 $_{drying}$ 0.3-3.5 |

These results indicate that the meatmeals which were produced by dry batch rendering contained the lowest total levels of biogenic amines. The meatmeals produced by digestor wet rendering and low temperature rendering also had low levels of total biogenic amines however it is difficult to draw a conclusion as these two processes had a small number of samples analysed. Further sampling of meatmeals from digestor wet rendering and low temperature rendering plants is required to obtain more conclusive results.

## Protein and Ash

The samples were analysed for protein and ash contents (Tables 2.1-2.4). The protein content of samples produced by dry batch rendering ranged from 42-62%. The ash content ranged from 10-39%. Samples produced by continuous dry rendering had protein levels between 47-63% and ash contents between 18-39%. The protein levels and ash contents of samples produced by digestor wet rendering ranged from 46-47% and 12-38%, respectively. Low temperature rendered samples had protein levels between 50-58% and ash content between 14-36%.

Table 2.1 DRY BATCH RENDERING

| Sample | Time delay (Hours) | Putrescine mg/kg | Cadaverine mg/kg | Histamine mg/kg | Total Amines mg/kg | Protein % | Ash % |
|---|---|---|---|---|---|---|---|
| 1 | 3 | 75 | 143 | 22 | 240 | 59 | 22 |
| 3 | 8-16 | 90 | 139 | 15 | 244 | 59 | 24 |
| 5A | 0.5-8 | 62 | 82 | 17 | 161 | 53 | 26 |
| 6 | 8-36 | 54 | 126 | 12 | 192 | 50 | 31 |
| 10A | 2.5 | 20 | 18 | 28 | 66 | 49 | 38 |
| 10B | 11 | 129 | 324 | 41 | 494 | 51 | 33 |
| 15 | 0.75 | 14 | 14 | 19 | 47 | 47 | 37 |
| 18 | 10 | 86 | 96 | 32 | 214 | 66 | 10 |
| 19 | 0.5 | 11 | 9 | 15 | 35 | 43 | 38 |
| 20A | 0.5 | 15 | 19 | 10 | 44 | 48 | 38 |
| 20B | 2 | 12 | 8 | 18 | 38 | 47 | 38 |
| 20C | 8 | 16 | 19 | 13 | 48 | 52 | 32 |
| 22 | 4 | 22 | 23 | 24 | 69 | 48 | 33 |
| 26 | 6 | 23 | 32 | 10 | 65 | 48 | 35 |
| 29 | 2 | 11 | 14 | 15 | 40 | 45 | 34 |
| 31 | 0 | 19 | 31 | 30 | 80 | 57 | 16 |
| 32 | 5 | 22 | 40 | 12 | 74 | 50 | 21 |
| 36 | 4 | 50 | 57 | 32 | 139 | 50 | 16 |
| 37A | 6-12 | 18 | 56 | 31 | 105 | 51 | 31 |
| 37B | 1 | 14 | 17 | 18 | 49 | 44 | 35 |
| 38A | 4 | 10 | 3 | 18 | 31 | 58 | 20 |
| 38B | 4 | 11 | 8 | 11 | 30 | 56 | 23 |
| 39 | 4 | 75 | 92 | 31 | 198 | 49 | 31 |
| 41A | 0 | 19 | 31 | 9 | 59 | 54 | 26 |
| 41B | 0 | 18 | 17 | 31 | 66 | 55 | 22 |
| 41C | 0 | 13 | 21 | 21 | 55 | 50 | 32 |
| 41D | 0 | 9 | 28 | 7 | 44 | 54 | 26 |
| 41E | 0 | 2 | 7 | 11 | 20 | 53 | 25 |
| 41F | 0 | 22 | 13 | 24 | 59 | 61 | 22 |
| 41G | 0 | 15 | 17 | 15 | 47 | 53 | 27 |
| 41H | 0 | 13 | 18 | 8 | 39 | 53 | 28 |
| 41I | 0 | 9 | 14 | 5 | 28 | 44 | 38 |
| 41J | 0 | 12 | 10 | 14 | 36 | 52 | 27 |
| 41K | 0 | 15 | 13 | 22 | 50 | 53 | 24 |
| 41L | 0 | 13 | 16 | 13 | 42 | 46 | 32 |
| 41M | 0 | 10 | 11 | 10 | 31 | 47 | 33 |
| 41N | 0 | 9 | 13 | 6 | 28 | 45 | 36 |
| 41O | 0 | 21 | 18 | 27 | 66 | 61 | 16 |
| 41P | 0 | 11 | 19 | 8 | 38 | 51 | 31 |
| 41Q | 0 | 7 | 13 | 6 | 26 | 57 | 27 |
| 41R | 0 | 16 | 12 | 12 | 40 | 59 | 22 |
| 41S | 0 | 23 | 10 | 20 | 53 | 58 | 24 |
| 41T | 0 | 27 | 7 | 27 | 61 | 57 | 26 |
| 41U | 0 | 24 | 33 | 16 | 73 | 53 | 24 |
| 41V | 0 | 14 | 17 | 6 | 37 | 43 | 39 |
| 43 | 12-18 | 44 | 62 | 13 | 119 | 42 | 34 |
| 44 | 4-14 | 46 | 53 | 29 | 128 | 52 | 27 |

Table 2.2 CONTINUOUS DRY RENDERING

| Sample | Time delay (Hours) | Putrescine mg/kg | Cadaverine mg/kg | Histamine mg/kg | Total Amines mg/kg | Protein % | Ash % |
|---|---|---|---|---|---|---|---|
| 5B | 0.5-8 | 88 | 112 | 29 | 229 | 50 | 26 |
| 7 | 4 | 11 | 17 | 21 | 49 | 51 | 36 |
| 8 | 2 | 23 | 28 | 15 | 66 | 56 | 26 |
| 12 | 4 | 13 | 19 | 7 | 39 | 54 | 29 |
| 13 | 1 | 23 | 13 | 31 | 67 | 50 | 29 |
| 14 | 10 | 56 | 70 | 13 | 139 | 56 | 27 |
| 16 | 10 | 26 | 43 | 8 | 77 | 53 | 35 |
| 17A | 24 | 293 | 329 | 34 | 656 | 59 | 20 |
| 17B | 24 | 71 | 160 | 19 | 250 | 53 | 26 |
| 17C | 24 | 162 | 261 | 28 | 451 | 60 | 21 |
| 17D | 3 | 137 | 202 | 25 | 364 | 63 | 18 |
| 17E | 3 | 41 | 67 | 16 | 124 | 49 | 34 |
| 17F | 3 | 57 | 89 | 16 | 162 | 49 | 36 |
| 17G | 6 | 59 | 87 | 16 | 162 | 45 | 39 |
| 17H | 6 | 55 | 94 | 21 | 170 | 55 | 28 |
| 17I | 6 | 82 | 149 | 16 | 247 | 56 | 27 |
| 17J | - | 70 | 89 | 19 | 178 | 49 | 32 |
| 17K | - | 52 | 77 | 18 | 147 | 50 | 33 |
| 17L | - | 55 | 100 | 14 | 169 | 52 | 31 |
| 24 | 4-6 | 7 | 6 | 23 | 36 | 47 | 37 |
| 28 | 12-16 | 26 | 33 | 24 | 83 | 51 | 32 |
| 30 | 0-6 | 49 | 78 | 20 | 147 | 54 | 29 |
| 34 | 2 | 21 | 39 | 29 | 89 | 50 | 40 |
| 40 | 3 | 53 | 46 | 22 | 121 | 52 | 31 |
| 45 | 2 | 35 | 75 | 21 | 131 | 59 | 23 |
| 46 | 0.5 | 16 | 19 | 15 | 50 | 46 | 32 |
| 47 | 3 | 10 | 13 | 11 | 34 | 51 | 31 |

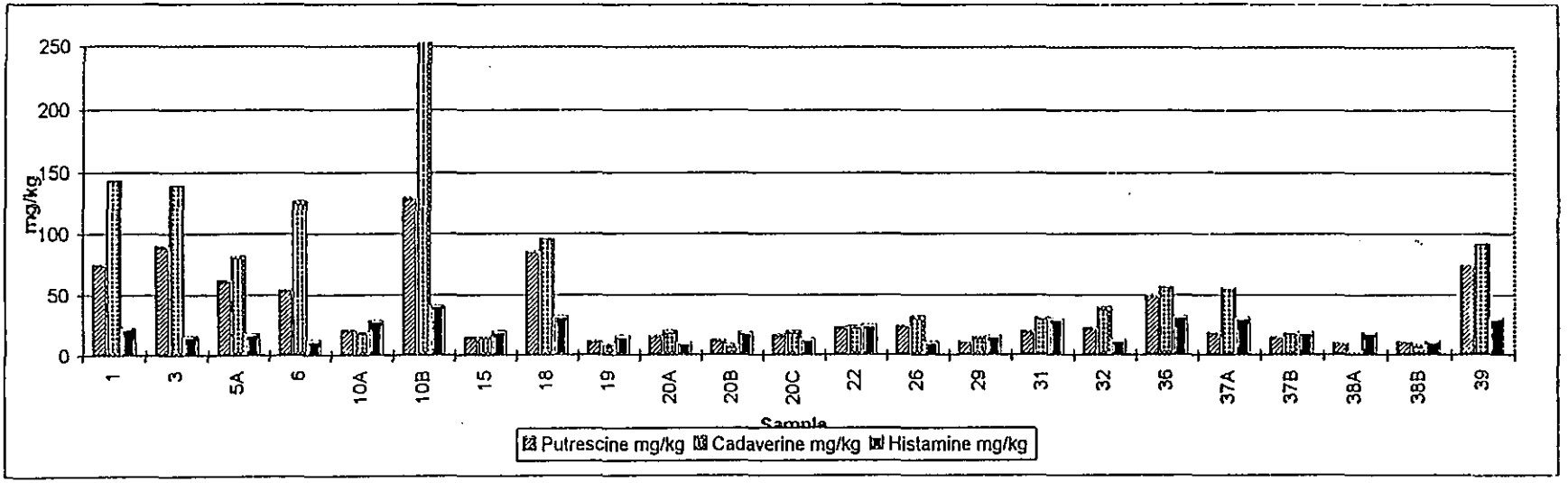
Table 2.3 DIGESTOR WET RENDERING

| Sample | Time delay (Hours) | Putrescine mg/kg | Cadaverine mg/kg | Histamine mg/kg | Total Amines mg/kg | Protein % | Ash % |
|---|---|---|---|---|---|---|---|
| 11 | 2 | 56 | 29 | 42 | 127 | 47 | 12 |
| 23 | 20 | 31 | 43 | 18 | 92 | 46 | 35 |
| 35 | 1.5 | 17 | 16 | 18 | 51 | 47 | 38 |

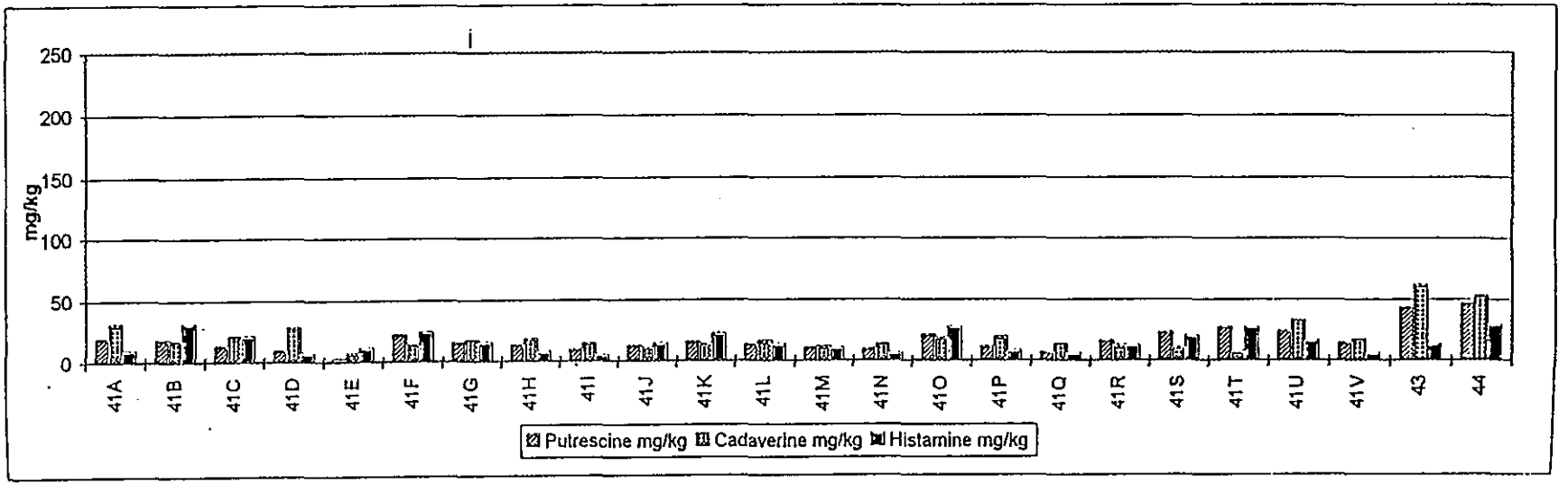
Table 2.4 LOW TEMPERATURE RENDERING

| Sample | Time delay (Hours) | Putrescine mg/kg | Cadaverine mg/kg | Histamine mg/kg | Total Amines mg/kg | Protein % | Ash % |
|---|---|---|---|---|---|---|---|
| 2 | 1 | 39 | 39 | 10 | 88 | 50 | 36 |
| 9 | 2 | 28 | 31 | 22 | 81 | 52 | 27 |
| 21 | 6 | 9 | 6 | 13 | 28 | 52 | 35 |
| 42 | <36 | 350 | 430 | 58 | 838 | 58 | 14 |

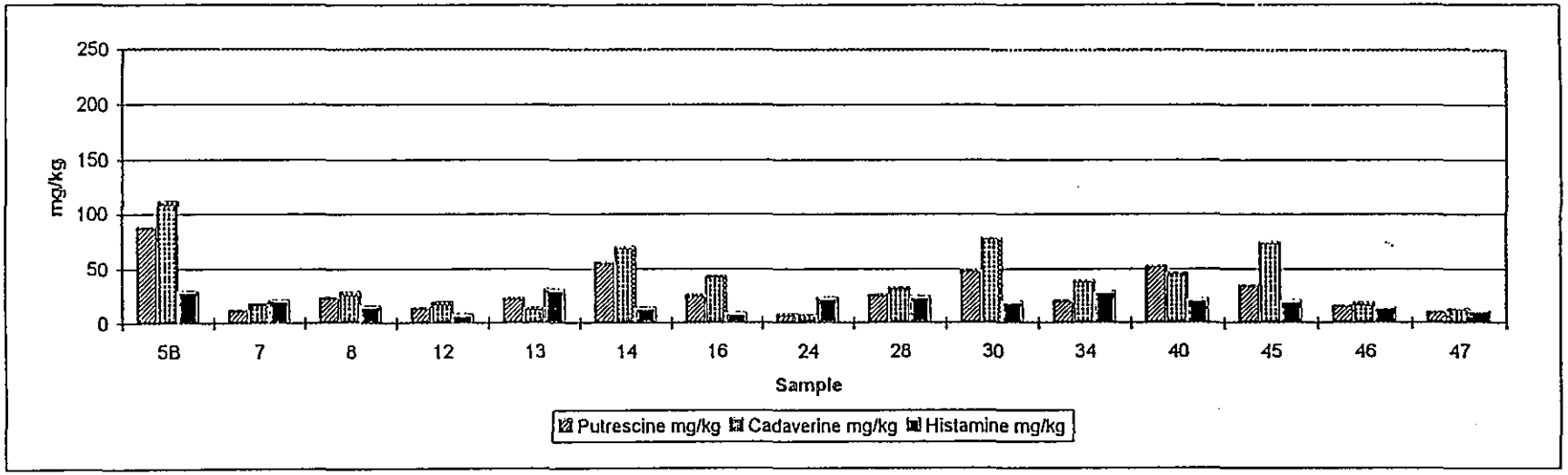
Graph 2.1 DRY BATCH RENDERING



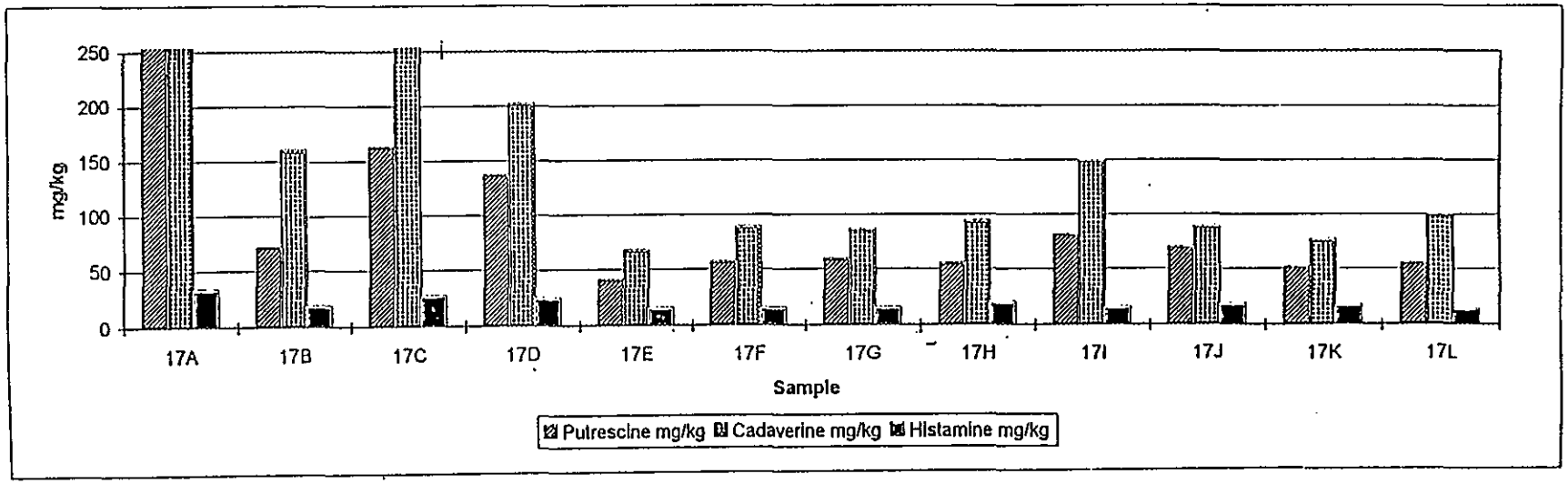

Graph 2.2 DRY BATCH RENDERING



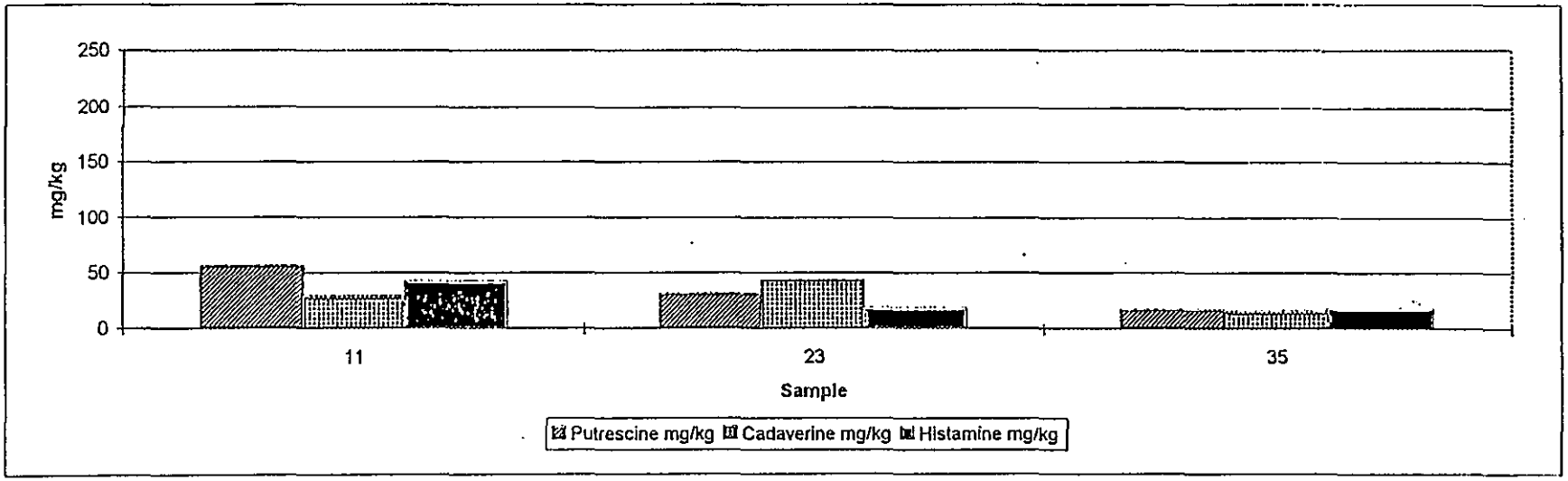
Graph 2.3 CONTINUOUS DRY RENDERING



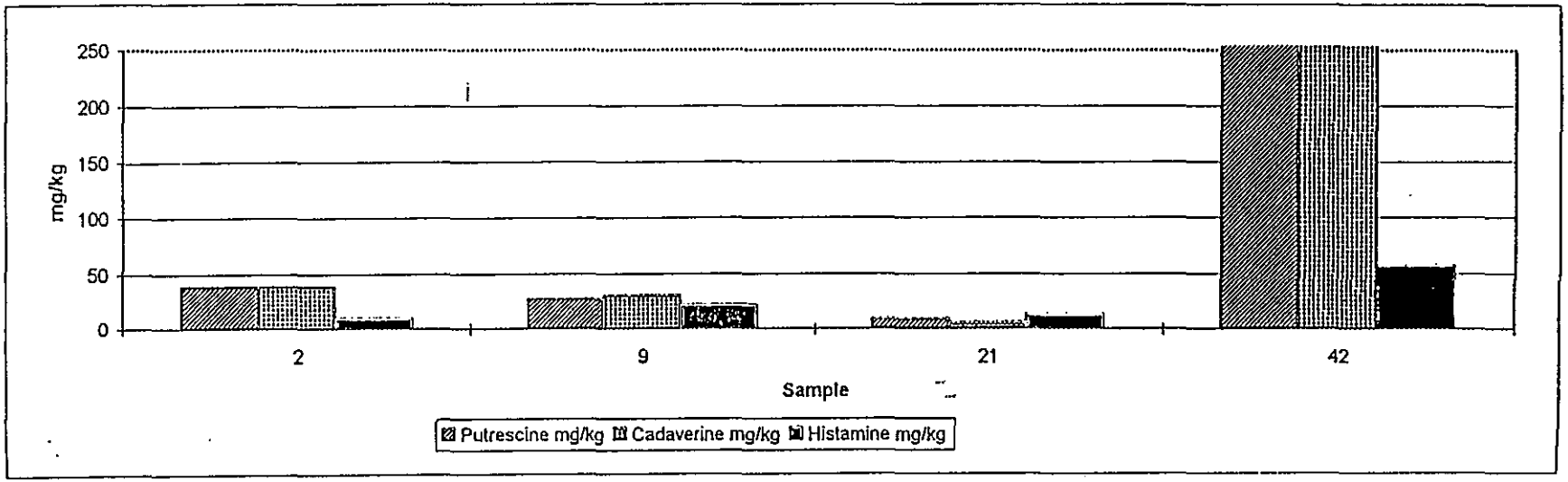

Graph 2.4 CONTINUOUS DRY RENDERING



Graph 2.5 DIGESTOR WET RENDERING




Graph 2.6 LOW TEMPERATURE RENDERING



Appendix 1

## QUESTIONNAIRE

1a. What age was the raw material (after kill) when it was received at the rendering plant?

---

1b. How many hours was the raw material in transit?

---

2. Was the raw material transported from the abattoir to the rendering plant under refrigerated conditions? (Tick correct box).

☐ ☐

Yes, Temp = _____°C No _____

3a. What was the approximate composition of the raw material when received? (eg: 60% Beef, 30% Mutton, 10% Pork)

---

3b. Were the digestive tract organs present in the raw material? (Tick correct box).

☐ ☐

Yes _____ No _____

3c. Was the particle size of the raw material reduced before rendering commenced?

If yes, please indicate the time prior to rendering

Yes, Time = _____ No _____

4a. Once the raw material was received, what length of time passed before rendering was commenced?

---

4b. During this time at what temperature was the raw material kept?

---

5a. What rendering process was used to produce the meatmeal or meat and bone meal? (Tick correct box)

| | | | |
|---|---|---|---|
| A: | Digestor wet rendering | E: | Low temperature rendering |
| B: | Dry batch rendering | F: | Ultra low temperature rendering |
| C: | Continuous dry rendering | G: | Other |
| D: | Semi continuous dry rendering | | |

5b. Can you give a brief outline of the process?

---

5c: What was the operating temperature and pressure of the rendering system?

---

5d. How long was the material cooked for?

---

6a. What were the specifications of the processed meatmeal or meat and bone meal?
(eg: 50% protein, 5% fat)

---

6b. Were there any control measures to ensure the specifications were met?

---

# 3. Survey of Selected Renders

Twenty one manufacturers completed a questionnaire (Appendix 2) and participated supplying meatmeal samples from dry batch, continuous dry, digestor wet, semi continuous, low temperature and high temperature rendering systems.

This survey is ongoing and results to date follow (Tables 3.1-3.5; Graphs 3.1-3.6). It can be noted that there is a large batch to batch variation within a rendering system.

Table 3.1 DRY BATCH RENDERING

| Sample | Date Sampled | Putrescine mg/kg | Cadaverine mg/kg | Histamine mg/kg | Total amines mg/kg |
|---|---|---|---|---|---|
| 3A | 6/2/96 | 193 | 187 | 36 | 416 |
| 3B | 21/2/96 | 61 | 92 | 14 | 167 |
| 3C | 6/3/96 | 201 | 324 | 33 | 558 |
| 3D | 19/3/96 | 49 | 98 | 20 | 167 |
| 3E | 26/3/96 | 90 | 141 | 18 | 249 |
| 3F | 2/4/96 | 40 | 62 | 8 | 110 |
| 3G | 16/4/96 | 32 | 41 | 10 | 83 |
| 19A | 12/12/95 | 57 | 42 | 19 | 118 |
| 19B | 22/12/95 | 24 | 11 | 12 | 47 |
| 19C | 10/1/96 | 21 | 10 | 16 | 47 |
| 19D | 15/3/96 | 74 | 63 | 18 | 155 |
| 20A | 6/2/96 | 69 | 90 | 11 | 170 |
| 20B | 15/3/96 | 34 | 39 | 8 | 81 |
| 20C | 15/4/96 | 52 | 59 | 8 | 119 |
| 26A | 7/2/96 | 29 | 40 | 10 | 79 |
| 31A | 20/2/96 | 40 | 62 | 20 | 122 |
| 31B | 6/3/96 | 28 | 33 | 15 | 76 |
| 31C | 20/3/96 | 23 | 31 | 14 | 68 |
| 36A | 8/12/95 | 64 | 88 | 29 | 181 |
| 36B | 26/2/96 | 190 | 233 | 42 | 465 |
| 36D | 15/3/96 | 138 | 196 | 32 | 366 |
| 36E | 25/3/96 | 97 | 89 | 22 | 208 |
| 37Ai | 7/12/95 | 33 | 50 | 21 | 104 |
| 37Aii | 18/12/96 | 30 | 42 | 23 | 95 |
| 37Aiii | 25/1/96 | 25 | 25 | 22 | 72 |
| 37Aiv | 23/2/96 | 37 | 47 | 21 | 105 |
| 37Bi | 23/1/96 | 26 | 53 | 45 | 124 |
| 37Bii | 8/2/96 | 7 | 6 | 21 | 34 |
| 37Biii | 22/2/96 | 9 | 7 | 28 | 44 |
| 37Biv | 14/3/96 | 41 | 74 | 28 | 143 |
| 37Bv | 21/3/96 | 20 | 23 | 27 | 70 |
| 37Bvi | 9/4/96 | 30 | 63 | 27 | 120 |

Table 3.2 DRY BATCH RENDERING

| Company | Date | Putrescine mg/kg | Cadaverine mg/kg | Histamine mg/kg | Total amines mg/kg |
|---|---|---|---|---|---|
| 41A | 3/1/96 | 17 | 12 | 21 | 50 |
| 41B | 17/1/96 | 16 | 23 | 19 | 58 |
| 41C | 1/2/96 | 19 | 18 | 30 | 67 |
| 41D | 15/2/96 | 20 | 19 | 17 | 56 |
| 41E | 29/2/96 | 13 | 9 | 13 | 35 |
| 41F | 14/3/96 8 | 15 | 12 | 19 | 46 |
| 41G | 18/3/96 1 | 30 | 31 | 31 | 92 |
| 41H | 18/3/96 2 | 25 | 24 | 19 | 68 |
| 41I | 18/3/96 3 | 20 | 41 | 39 | 100 |
| 41J | 18/3/96 4 | 14 | 15 | 14 | 43 |
| 41K | 18/3/96 5 | 13 | 11 | 9 | 33 |
| 41L | 19/3/96 6 | 44 | 39 | 16 | 99 |
| 41M | 19/3/96 7 | 23 | 27 | 9 | 59 |
| 41N | 19/3/96 8 | 19 | 16 | 12 | 47 |
| 41O | 19/3/96 9 | 18 | 14 | 24 | 56 |
| 41P | 19/3/96 10 | 24 | 26 | 21 | 71 |
| 41Q | 19/3/96 11 | 29 | 21 | 15 | 65 |
| 41R | 19/3/96 12 | 19 | 9 | 12 | 40 |
| 41S | 19/3/96 13 | 14 | 5 | 15 | 34 |
| 41T | 19/3/96 14 | 15 | 8 | 5 | 28 |
| 41U | 19/3/96 15 | 11 | 7 | 5 | 23 |
| 41V | 19/3/96 16 | 23 | 11 | 26 | 60 |
| 41W | 19/3/96 17 | 20 | 9 | 17 | 46 |
| 41X | 19/3/96 18 | 19 | 9 | 19 | 47 |
| 41Y | 19/3/96 19 | 36 | 62 | 20 | 118 |
| 41Z | 19/3/96 20 | 25 | 46 | 12 | 83 |
| 41A1 | 19/3/96 21 | 44 | 32 | 18 | 94 |
| 41B1 | 19/3/96 22 | 19 | 24 | 9 | 52 |
| 41C1 | 19/3/96 23 | 12 | 5 | 13 | 30 |

Table 3.3 CONTINUOUS DRY RENDERING

| Sample | Date Sampled | Putrescine mg/kg | Cadaverine mg/kg | Histamine mg/kg | Total amines mg/kg |
|---|---|---|---|---|---|
| 7A | 6/12/95 | 18 | 25 | 20 | 63 |
| 7B | 21/2/96 | 27 | 37 | 19 | 83 |
| 7C | 15/3/96 | 21 | 28 | 24 | 73 |
| 12A | 12/12/95 | 23 | 32 | 8 | 63 |
| 12B | 29/12/95 | 19 | 32 | 8 | 59 |
| 12C | 5/2/96 | 16 | 27 | 6 | 49 |
| 12D | 26/2/96 | 22 | 37 | 9 | 68 |
| 12E | 4/3/96 | 27 | 40 | 10 | 77 |
| 12F | 12/3/96 | 31 | 43 | 10 | 84 |
| 12G | 18/3/96 | 20 | 28 | 8 | 56 |
| 12H | 26/3/96 | 15 | 21 | 7 | 43 |
| 12I | 15/4/96 | 12 | 15 | 7 | 34 |
| 14A | 1/2/96 | 126 | 164 | 21 | 311 |
| 30A | 7/12/95 | 220 | 185 | 19 | 424 |
| 30B | 7/2/96 | 142 | 152 | 18 | 312 |
| 30C | 20/2/96 | 130 | 145 | 19 | 294 |
| 30D | 11/3/96 | 108 | 119 | 17 | 244 |
| 30E | 25/3/96 | 87 | 90 | 14 | 191 |
| 30F | 11/4/96 | 92 | 98 | 24 | 214 |
| 28A | 14/12/95 | 12 | 10 | 26 | 48 |
| 28B | 15/1/96 | 46 | 53 | 11 | 110 |
| 28C | 27/3/96 | 21 | 14 | 18 | 53 |
| 34A | 11/12/95 | 58 | 88 | 23 | 169 |
| 34B | 5/1/96 | 33 | 45 | 30 | 108 |
| 34C | 22/1/96 | 48 | 69 | 28 | 145 |
| 34D | 14/3/96 | 46 | 60 | 23 | 129 |
| 46A | 15/2/96 | 23 | 28 | 16 | 67 |

Table 3.4 DIGESTOR WET RENDERING

| Sample | Date Sampled | Putrescine mg/kg | Cadaverine mg/kg | Histamine mg/kg | Total Amines mg/kg |
|---|---|---|---|---|---|
| 23A | 27/2/96 | 15 | 16 | 13 | 44 |
| 23B | 14/3/96 | 17 | 18 | 19 | 54 |
| 23C | 9/3/96 | 35 | 23 | 12 | 70 |
| 23D | 28/3/96 | 12 | 6 | 12 | 30 |
| 35A | 12/12/95 | 78 | 149 | 10 | 237 |

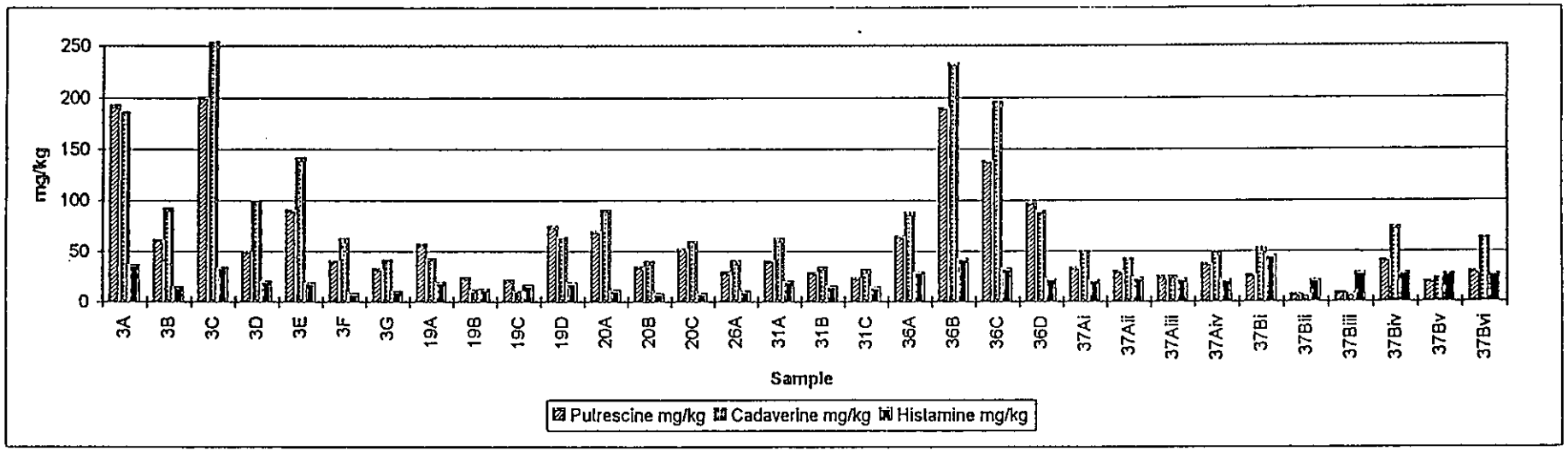
Table 3.4 LOW TEMPERATURE RENDERING

| Date | Date Sampled | Putrescine mg/kg | Cadaverine mg/kg | Histamine mg/kg | Total Amines mg/kg |
|---|---|---|---|---|---|
| 9A | 20/2/96 | 45 | 59 | 19 | 123 |
| 9B | 5/3/96 | 57 | 79 | 27 | 163 |
| 9C | 20/3/96 | 31 | 38 | 28 | 97 |
| 9D | 4/4/96 | 13 | 7 | 15 | 35 |
| 9E | 18/4/96 | 14 | 9 | 13 | 36 |
| 21A | 15/3/96 | 13 | 10 | 12 | 35 |
| 21B | 4/4/96 | 8 | 4 | 12 | 24 |
| 42A1 | 28/2/96 | 880 | 815 | 81 | 1776 |
| 42A2 | 29/2/96 | 620 | 613 | 61 | 1294 |
| 42A3 | 1/3/96 | 349 | 402 | 47 | 798 |
| 42A4 | 4/3/96 | 182 | 261 | 32 | 475 |
| 42A5 | 5/3/96 | 82 | 95 | 16 | 193 |
| 42A6 | 6/3/96 | 691 | 689 | 53 | 1433 |
| 42A7 | 7/3/96 | 757 | 735 | 55 | 1547 |
| 42A8 | 8/3/96 | 417 | 557 | 41 | 1015 |
| 42A9 | 11/3/96 | 415 | 523 | 47 | 985 |
| 42A10 | 13/3/96 | 504 | 617 | 63 | 1184 |

Table 3.5 HIGH TEMPERATURE RENDERING

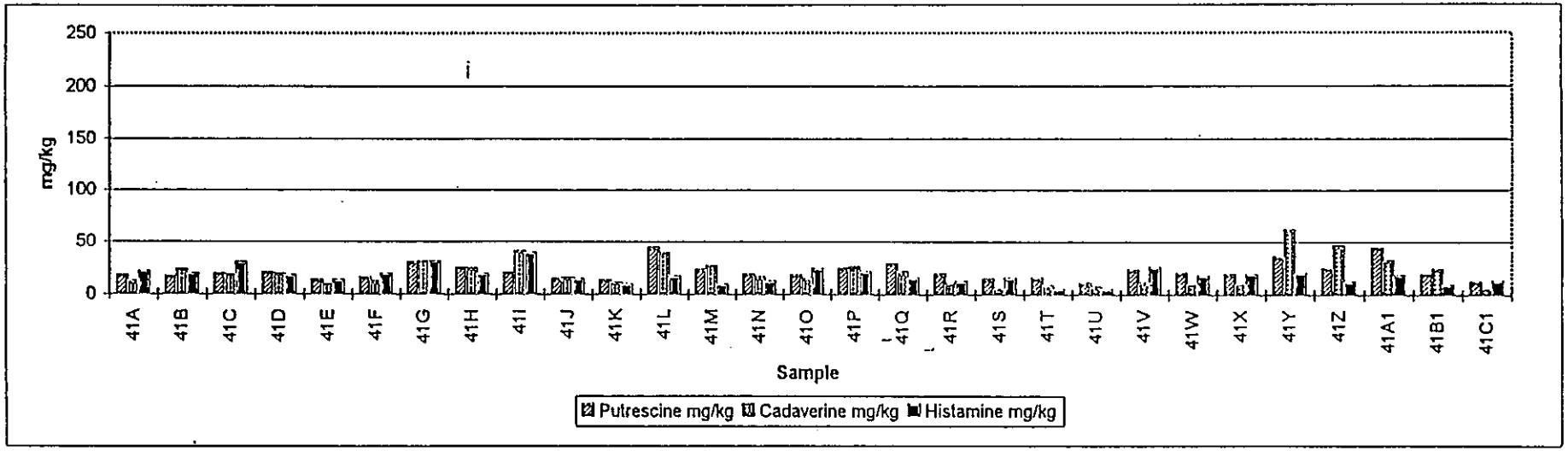
| Date | Date Sampled | Putrescine mg/kg | Cadaverine mg/kg | Histamine mg/kg | Total Amines mg/kg |
|---|---|---|---|---|---|
| 42B1 | 28/2/96 | 340 | 383 | 21 | 744 |
| 42B2 | 29/2/96 | 90 | 110 | 12 | 212 |
| 42B3 | 1/3/96 | 95 | 122 | 15 | 232 |
| 42B4 | 4/3/96 | 61 | 75 | 15 | 151 |
| 42B5 | 5/3/96 | 212 | 230 | 29 | 471 |
| 42B6 | 6/3/96 | 186 | 220 | 16 | 422 |
| 42B7 | 7/3/96 | 53 | 73 | 11 | 137 |
| 42B8 | 8/3/96 | 213 | 258 | 23 | 494 |
| 42B9 | 11/3/96 | 94 | 121 | 16 | 231 |
| 42B10 | 13/3/96 | 387 | 362 | 22 | 771 |

Graph 3.1 DRY BATCH RENDERING

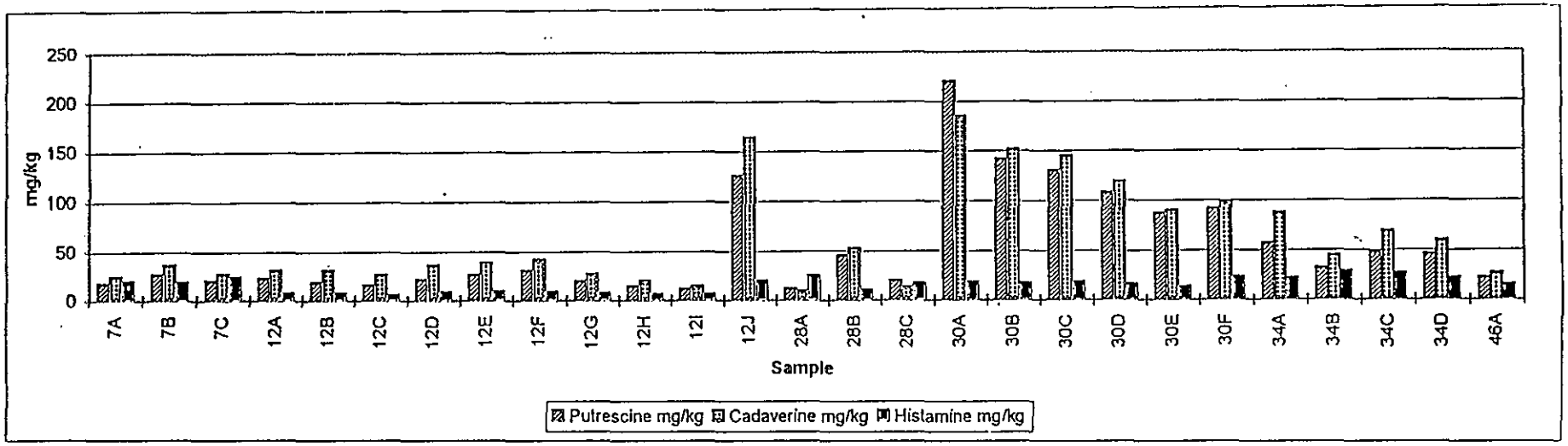




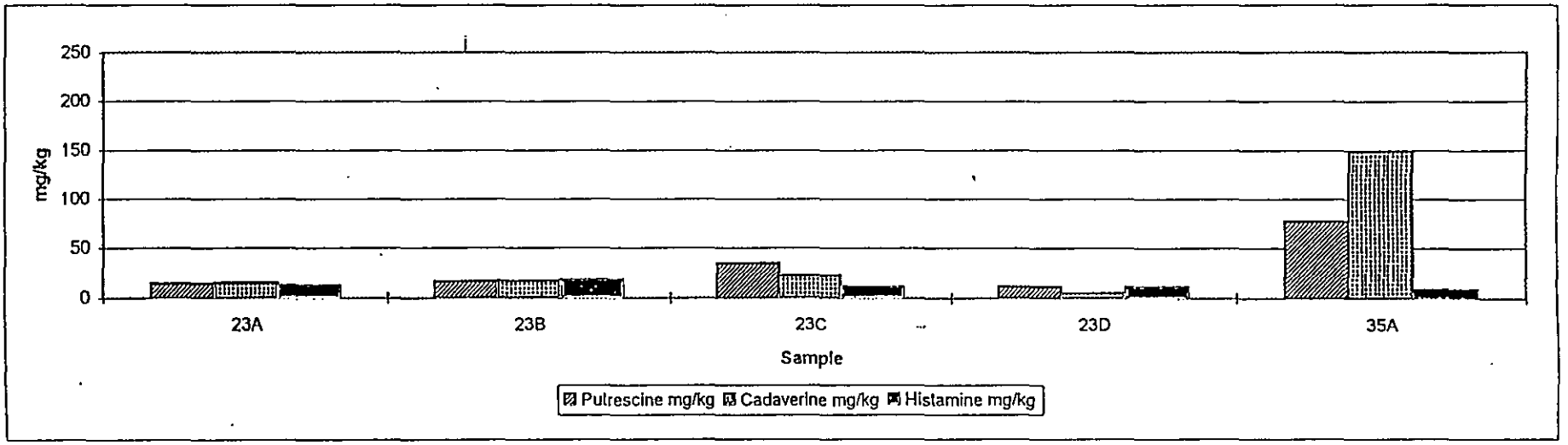

Graph 3.2 DRY BATCH RENDERING



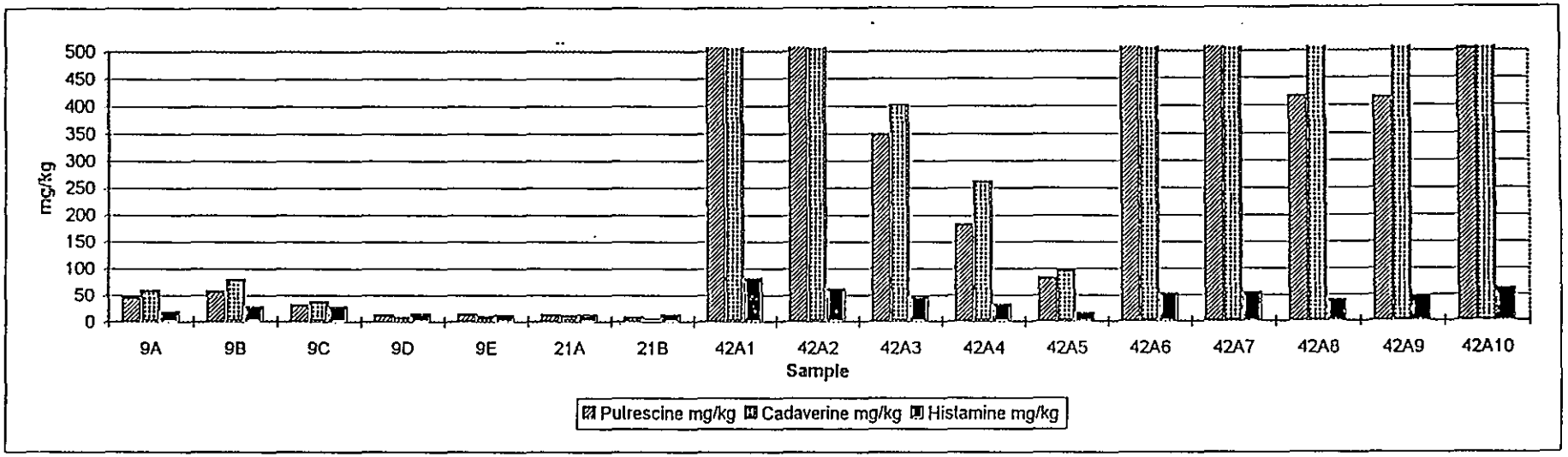
Graph 3.3 CONTINUOUS DRY RENDERING




Graph 3.4 DIGESTOR WET RENDERING

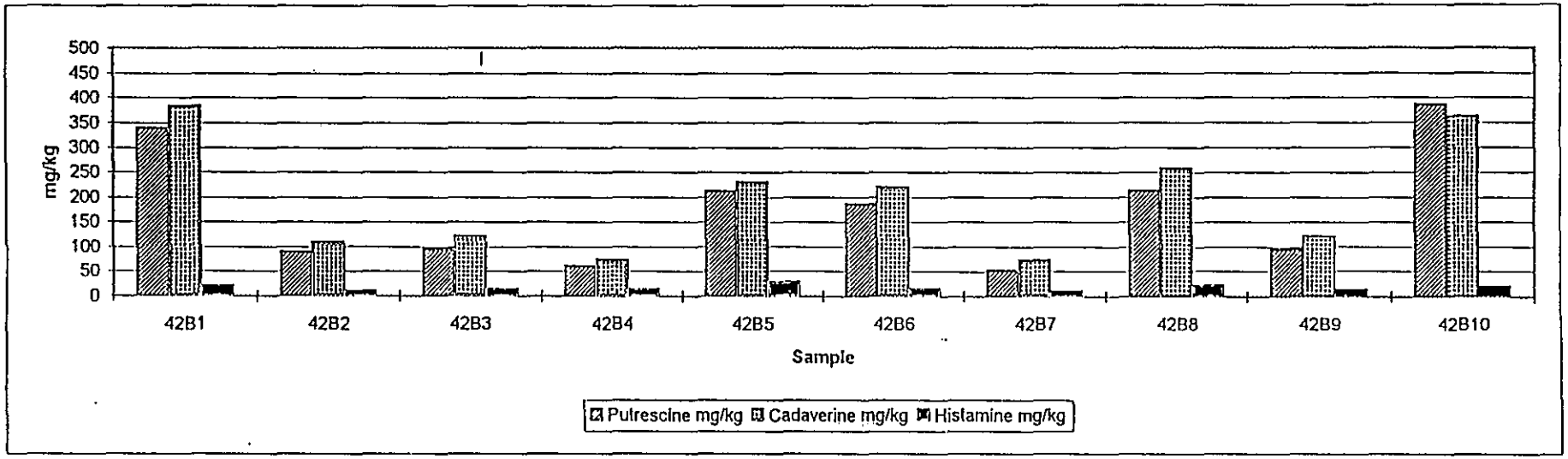


Graph 3.5 LOW TEMPERATURE RENDERING






Graph 3.6 HIGH TEMPERATURE RENDERING



# QUESTIONNAIRE

Company Name: _____

Date of sampling: _____

1a. What age was the raw material (after kill) when it was received at the rendering plant?

_____

1b How many hours was the raw material in transit?

_____

2. Was the raw material transported from the abattoir to the rendering plant under refrigerated conditions? (Tick correct box).

Yes, Temp = _____°C No _____

3a. What was the approximate composition of the raw material when received? (eg: 60% Beef, 30% Mutton, 10% Pig)

_____

3b. Were the digestive tract organs present in the raw material? (Tick correct box).

Yes, No _____

3c. Was the particle size of the raw material reduced before rendering commenced? If yes, please indicate the time prior to rendering

Yes, Time = _____ No _____

4a. Once the raw material was received, what length of time passed before rendering was commenced?

_____

4b. During this time at what temperature was the raw material kept?

_____

5a. How long was the material cooked for?

_____

6a. What were the specifications of the processed meatmeal or meat and bone meal? (eg: 50% protein, 5% fat)

_____

# 4. Pilot Planting Processing

## Preliminary Results

### Experiment 1

*Aim:*

To determine the effects of storage of raw materials and various heating regimes on the production of the biogenic amines putrescine, cadaverine and histamine.

*Method:*

Typical raw materials used in meatmeal production (bone, meat scraps) were given various heat treatments from under cooked (levels), fully rendered and over rendered. An industrial microwave at two energy levels was used in order to obtain consistent and even heat to all areas. Biogenic amines were determined by HPLC.

*Results:*

Storage of the raw materials produced a slight increase in biogenic amines with a large increase in cadaverine over 24 hours (Figure 1).

Rendering produced an initial increase in cadaverine and then a decrease in concentration. Overall there was a decrease in the levels of biogenic amines (Figures 2 and 3).

The actual levels of biogenic amines in the raw materials were very high because of the time delay in obtaining samples (24 hours post slaughter) which allows bacterial or enzyme production of amines and the second experiment was planned using fresh raw materials.

### Experiment 2

*Aim:*

To study the effects of different storage times and temperatures on biogenic amine production.

*Method:*

Fresh rendering raw material was obtained 1 hour after slaughter and subjected to storage times at 4° and 8°C (refrigeration and abattoir cool room). Biogenic amines were determined by HPLC.

*Results:*

The levels of biogenic amines in the raw materials was very low (Figure 4), which reflected the freshness of the raw material. Storage of the raw materials at 4°C maintained the histamine levels and decreased the cadaverine and putrescine levels. All of the values were well below acceptable limits. Histamine levels at 8°C were unchanged compared to 4°C but the levels of cadaverine and putrescine decreased markedly.

Rendering fresh material increased histamine levels but decreased cadaverine and putrescine (Figure 5). The meatmeal produced by rendering material stored at 8°C for 20 hours had a slightly elevated histamine content and a marked by increased cadaverine content while putrescine was unchanged.

A comparison of all of the meatmeals showed that the levels of biogenic amines were very low and storage of the raw materials before processing was important in controlling these levels (Figure 6).

However, in practice the raw materials may not be kept in the abattoir cool room and the delay in processing may be important in amine production.

Experiment 3 will study the effects of storage of raw materials at abattoir floor temperatures for 1, 2 and 3 days.

Figure 1

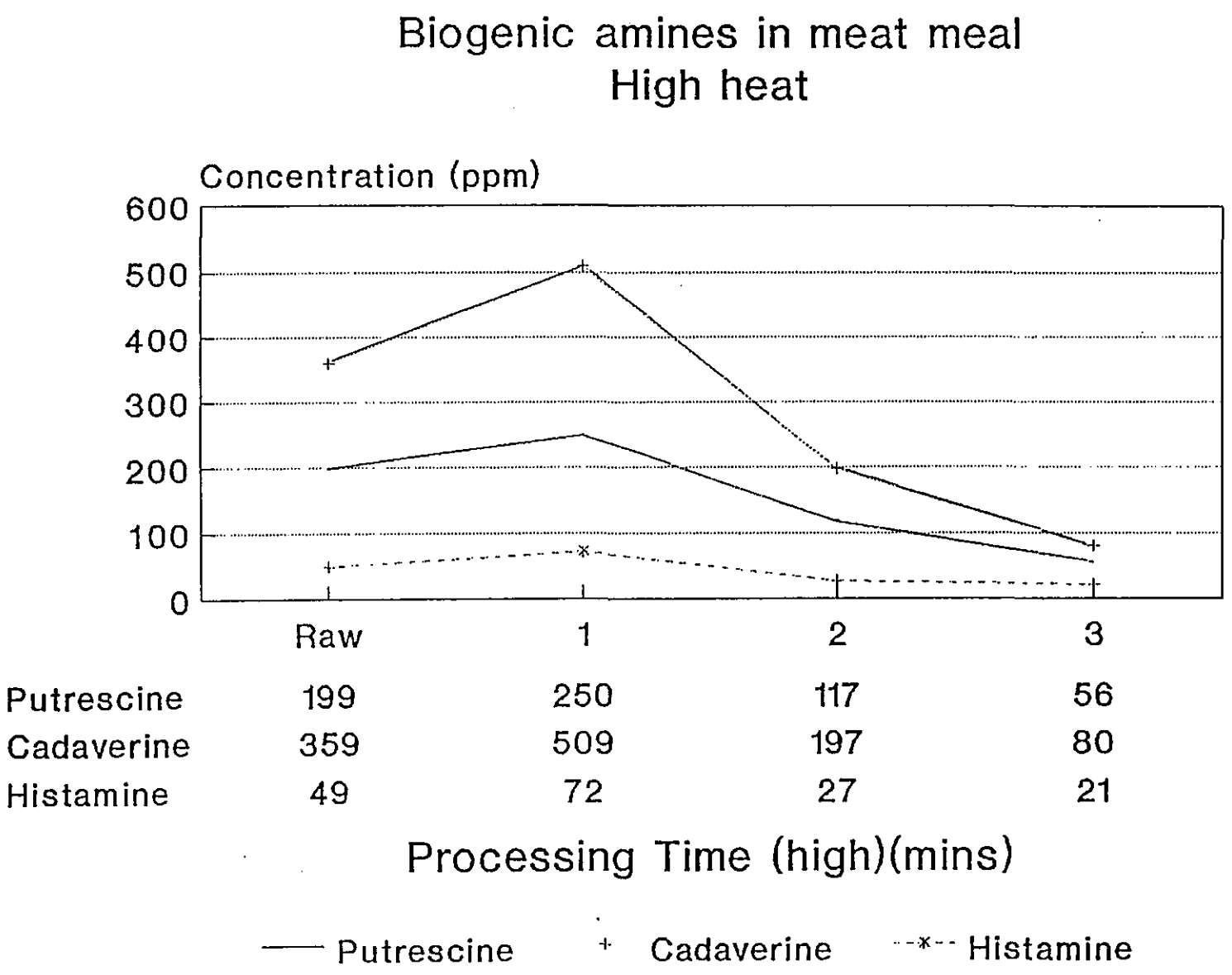

Biogenic amines in meat meal
High heat
Concentration (ppm)
600
500
400
300
200
100
0
Raw
1
2
3
Putrescine 199 250 117 56
Cadaverine 359 509 197 80
Histamine 49 72 27 21
Processing Time (high)(mins)
Putrescine
Cadaverine
Histamine

Figure 2

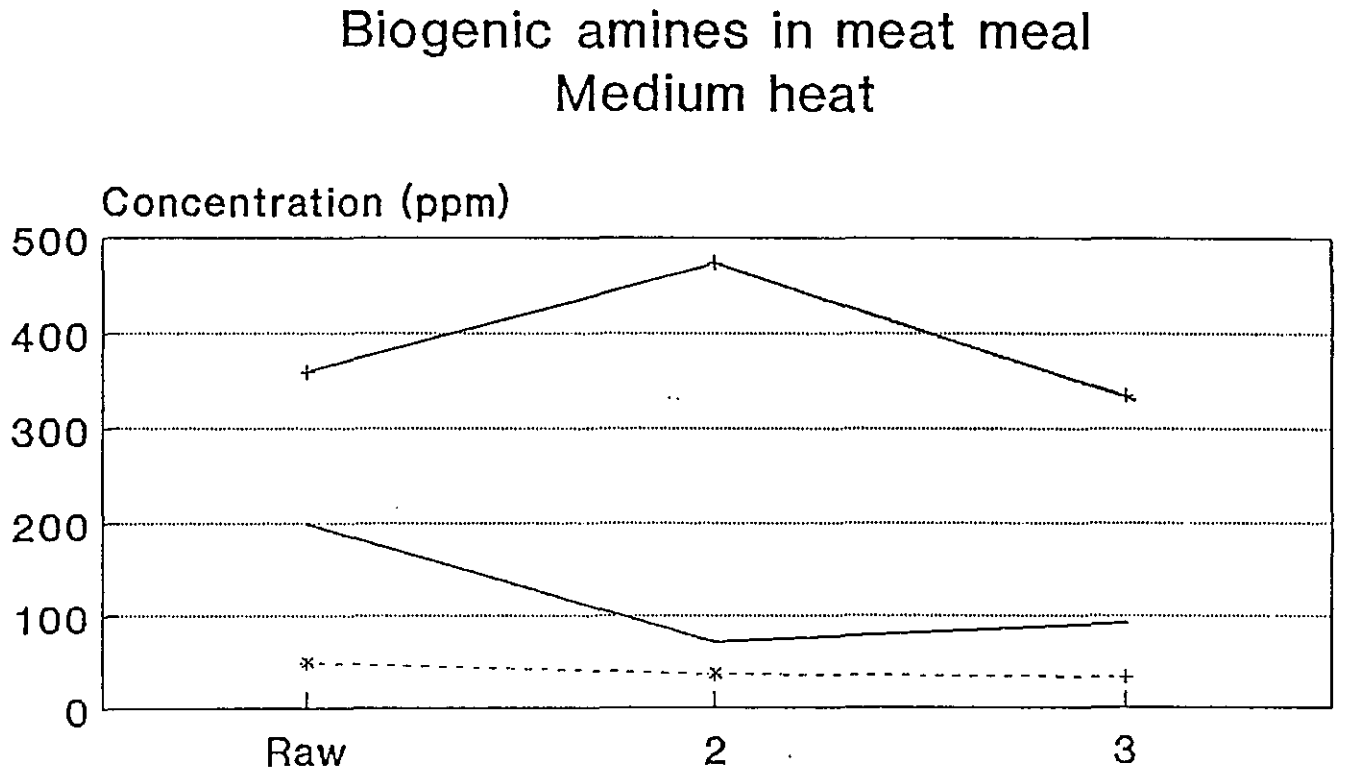

Biogenic amines in meat meal
Medium heat
Concentration (ppm)
500
400
300
200
100
0
Raw
2
3
Putrescine 199 72 92
Cadaverine 359 474 335
Histamine 49 36 33
Processing Time (high)(mins)
Putrescine
Cadaverine
Histamine

Figure 3

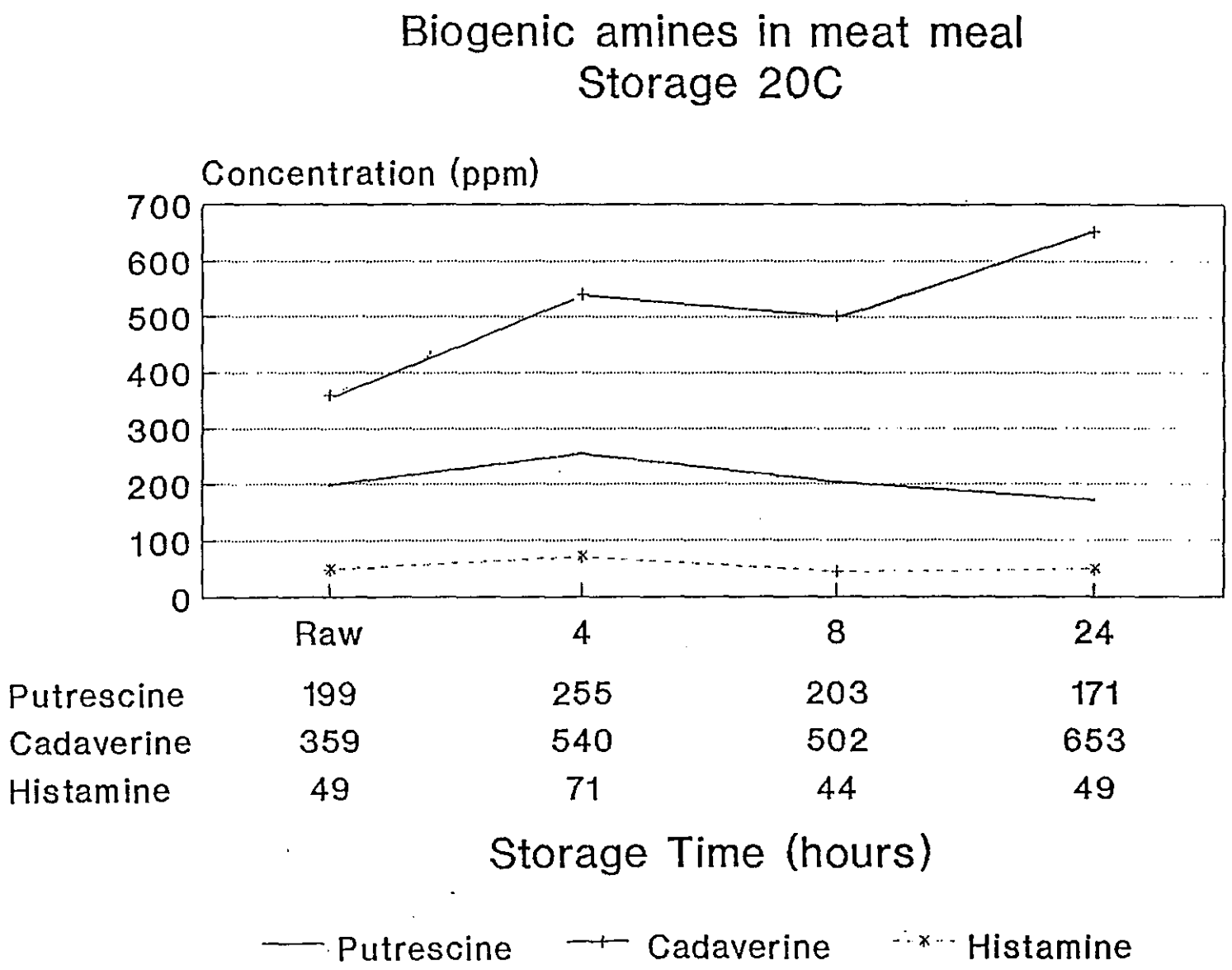

Biogenic amines in meat meal
Storage 20C
Concentration (ppm)
700
600
500
400
300
200
100
0
Raw
4
8
24
Putrescine 199 255 203 171
Cadaverine 359 540 502 653
Histamine 49 71 44 49
Storage Time (hours)
Putrescine
Cadaverine
Histamine

Figure 4

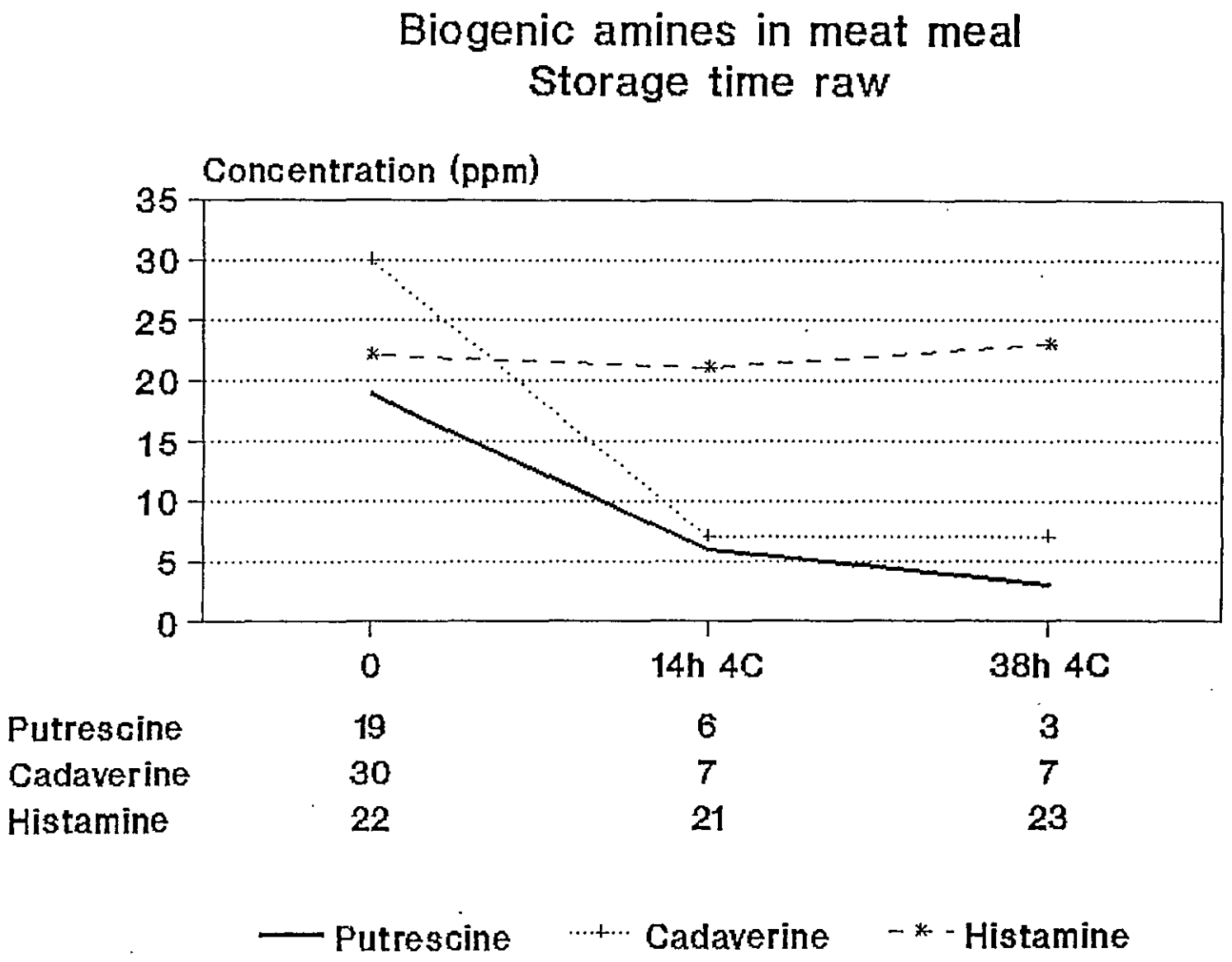
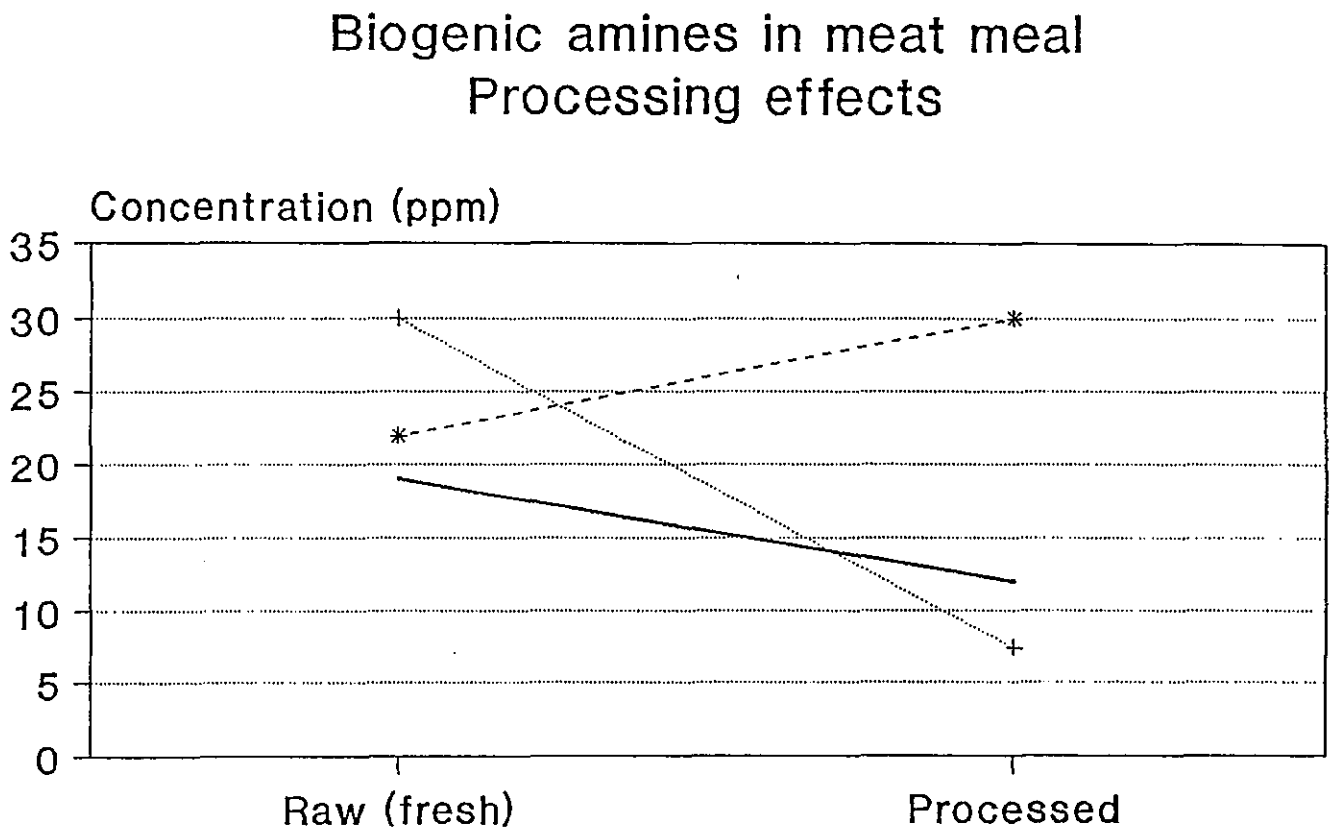
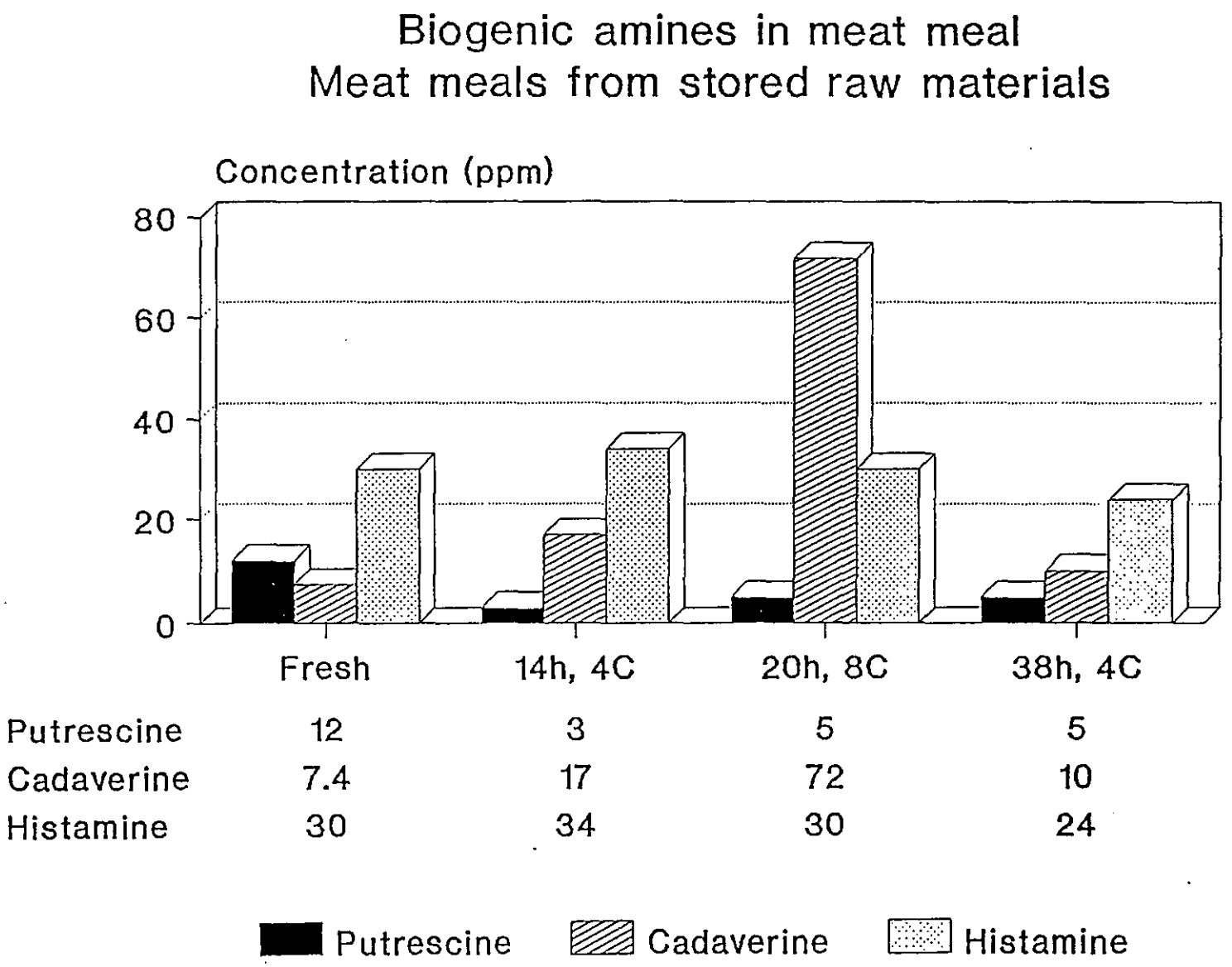

Biogenic amines in meat meal
Storage time raw
Concentration (ppm)
35
30
25
20
15
10
5
0
0
14h 4C
38h 4C
Putrescine 19 6 3
Cadaverine 30 7 7
Histamine 22 21 23
Putrescine
Cadaverine
Histamine

Figure 5




| | Raw (fresh) | Processed |
|---|---|---|
| Putrescine | 19 | 12 |
| Cadaverine | 30 | 7.4 |
| Histamine | 22 | 30 |

Figure 6


Biogenic amines in meat meal
Meat meals from stored raw materials
Concentration (ppm)
80
60
40
20
0
Fresh
14h, 4C
20h, 8C
38h, 4C
Putrescine 12 3 5 5
Cadaverine 7.4 17 72 10
Histamine 30 34 30 24
Putrescine
Cadaverine
Histamine