Institute of Health Care  
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DETERMINATION OF RESIDUAL LEVELS OF NITRITES AND NITRATES IN MEAT PRODUCTS  

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A Dissertation Submitted in Part Fulfilment
of the Requirements for
the Degree of Master of Health Science
(Environmental Health)

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June 1999
Declaration

I, hereby declare that
I have carried out this dissertation
and this is
entirely my own work.

Mario Fenech Caruana  B.Pharm.Tech.(Hons.)
Dedicated

to my beloved wife Felicienne,
to our darling daughter Tiziana,
to my parents & family,
and to all my sterling friends.
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ABSTRACT

Nitrite and nitrate occur naturally in food but may also be present as a result of the use of fertilisers on crops or from their use as preservatives. They are primarily added to processed meat products (in the form of curing preparations) to provide protection against microorganisms, particularly *Clostridium botulinum*, that can cause food poisoning.

Nitrite is considered as one of the most important food additives, both from an economical as well as from a technical point of view. Apart from exerting antibotulinal and other antimicrobial functions, addition of nitrite converts perishable meats, fish and poultry into unique cured products such as bacon and ham, having desirable sensory characteristics and a longer shelf-life. Nitrate, when added, serves mostly as a reservoir from which nitrite is derived. It is also added to certain foods (some meats, fish and dairy products) as a preservative but only becomes active when it is converted to nitrite via processing or microbial activity.

Both nitrite and nitrate are monitored regularly because of their toxicity. Their presence in food (especially meat products) may cause methaemoglobinaemia and favour the formation of carcinogenic nitrosamines. Although nitrate is more stable and less toxic, it gives rise to concern because it is easily converted to nitrite by microbial action.

The use of nitrite and nitrate in meat products is locally controlled by the Permitted Food Additives Regulations, 1998 which virtually conforms with the European Directive 95/2/EC on food additives and various other international standards.

Nitrite can be partially recovered in the finished meat product as “residual nitrite” which represents the measurable/detectable part of the curing salt in the complex meat matrix. The remaining part of added nitrite disappears by combining with pigments or by undergoing other reactions.
This project includes a survey to determine the residual level of nitrite and nitrate in 50 samples of meat products available on the local retail market. Nitrites were determined spectrophotometrically by the diazotisation-coupling technique (modified Griess-Ilosvay reaction) while nitrates were reduced to nitrites by the cadmium reduction technique before being assayed spectrophotometrically as nitrites.

The amount of residual sodium nitrite found in the samples varied from 1.8 to 10.2 mg/kg in canned products, <0.3 to 11.0 mg/kg in refrigerated products and <0.3 to 55.9 mg/kg in frozen products (with respective means of 7.3, 5.3 and 8.8 mg/kg). On the other hand, nitrate levels varied from 18.4 to 57.7 mg/kg in canned products, 13.6 to 162.7 mg/kg in refrigerated products and 22.6 to 487.4 mg/kg in frozen products (corresponding to mean values of 31.8, 72.7 and 93.2 mg/kg respectively).

All samples analysed were found to be within the maximum permitted level for nitrite; however, one product (namely locally preserved collar bacon) was found to exceed considerably the current regulatory limit for nitrate. The case however merits further investigations and legal considerations given that the period of the survey happened to coincide with a transition phase in local regulations on the use of preservatives in foodstuffs, involving a change in maximum level permitted from a total of 500 mg/kg (nitrite + nitrate), in the case of bacon & ham products, to an absolute maximum of 250 mg/kg of nitrate (as from 01.01.99).

Comparing the findings from this survey with those from similar surveys, it becomes evident that amounts of added nitrite have decreased over the years (reducing risk from volatile nitrosamines), while nitrate has been practically phased out/substituted. In fact, it is believed that current residual nitrite is about one tenth the level present about 25 years ago. However, studies show that there is no complete and suitable alternative for nitrite, in spite of the continuously changing processing techniques and formulations for the vast selection of cured meats produced. On the other hand, a total lack of nitrite may indicate potential risk to the consumer's health if the food in question is infected with Cl. botulinum.
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Chapter 1

INTRODUCTION

1.1 Techniques of Food Preservation

Food preservation aims at preventing or minimizing chemical and microbiological spoilage, thereby prolonging the shelf-life of the product. Microbiological spoilage is suppressed either by killing microorganisms and then storing food in conditions where further infection is impossible, or by creating an environment which is not suitable for their proliferation.

Techniques of food preservation have been in operation since prehistoric times. Foods were traditionally preserved using methods involving heat, chilling, drying, fermenting and treatment with certain chemicals (such as sodium chloride). It was not until recent years that chemical additives started being used on an extensive scale and that modern techniques such as heat sterilisation and irradiation came also into operation (Fox & Cameron, 1983).

Apart from providing effective means of microbial control, techniques of food preservation must conserve, as far as possible, both the original characteristics as well as the nutritive value of the food.
1.2 Chemical Preservatives

Chemical preservatives are added to foodstuffs to prevent or retard both CHEMICAL and MICROBIOLOGICAL deterioration of food..

(i) CHEMICAL DETERIORATION of food is prevented by the addition of:

- **antioxidants** - to prevent autoxidation of pigments, flavours, lipids and vitamins;
- **antibrowning agents** - to prevent enzymatic and non-enzymatic browning reactions;
- **antistaling compounds** - to prevent any changes in texture.

(ii) MICROBIOLOGICAL DETERIORATION is mainly controlled by the use of **antimicrobial agents** (e.g. nitrite in curing preparations).

The US Food & Drug Administration (1979) excludes "common salt, sugars, vinegars, spices or oils extracted from spices, substances added to food by direct exposure thereof to wood smoke, or chemicals applied for their respective insecticidal or herbicidal properties" from its official definition of the term Chemical Preservative.
Choosing the right preservative or combination of preservatives is crucial in securing a reasonable shelf-life (both chemical and biological) of a food product. For example, it is estimated that the use of antioxidants may alone extend the shelf-life of some products by 200% (Branen, 1975). The shelf-life can be further extended using a combination of preservatives to control both chemical and microbiological deterioration.

The effectiveness of chemical preservatives depends on various parameters such as chemical structure, antimicrobial specificity, solubility (usually in water), partitioning, microbial type and count, pH and water activity (Branen, 1991).

1.3 **Nitrite and Nitrate in Cured Meat Products**

Nitrites and nitrates have been used for centuries to preserve or 'cure' meat to ensure a continuing supply of meat during periods when fresh meat is not available. It is strongly believed that the salt that was originally used to preserve meat was probably contaminated with saltpetre (potassium nitrate). It was only later (late 1800s) that the action of saltpetre was recognised as being instrumental in producing a characteristic flavour and pink coloration in meat products (Binkerd & Kolari, 1975).
Further (historical) landmarks in the history of meat curing are attributed to Polenski (1891) who observed that nitrate was reduced to nitrite by bacterial action, Lehman (1899) who established that pink colour of cured meat derived from nitrite and not nitrate, and Haldane (1901) who found that pink colour resulted from the reaction of nitric oxide (NO) with meat pigments.

The curing process today involves addition of nitrite and/or nitrate, salt and certain colour-fixing ingredients to meat. Other additives may be spices and seasonings (to impart characteristic properties to the product), phosphates and other reducing agents (e.g. ascorbate, erythorbate). Heating may also be involved in some stage of the curing process (Hotchkiss & Cassens, 1987).

It has now been established that nitrite rather than nitrate is the principal active ingredient in curing salt mixtures. Nitrite is also regarded as a multifunctional good additive in view of the numerous properties it imparts to meat. The main functions of nitrite in meat are (Gray et al., 1981):

i) an antimicrobial action - providing protection against growth and toxin production of \textit{C. botulinum}, and several spoilage organisms;

ii) the development of the characteristic cured meat colour;
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iii) the development of the typical cured meat aroma;
iv) potent antioxidant properties - eliminating the problem of warmed-over flavour.

Nitrate is less reactive than nitrite and seems to have no direct effects on the preservation of meat. Yet, capable of at least being partially converted to nitrite by microbiological and biochemical processes occurring in meat, nitrate is still employed as an ingredient in some commercial curing processes.

Unfortunately the continued use of nitrite as a curing agent has been questioned because of its involvement in the formation of N-nitroso compounds (some of which being carcinogenic substances) in cured meats. Worldwide efforts are still being made to eliminate the use of nitrate and reduce nitrite levels to the minimum possible without jeopardising the efficacy of the latter's basic functions.

1.4 Objectives of Project

One of the aims of this project was to delve into several aspects of meat preservation, with particular reference to the curing technique and discuss in some detail the antimicrobial, antioxidant and organoleptic functions of nitrite and nitrate, the latter acting
mainly as a reservoir for further generation of nitrite via microbiological reduction.

Another important task was to discuss in depth the toxicology of nitrates, nitrites and their reaction products, namely N-nitroso compounds, in view of the well-known or potential carcinogenic activity of the latter in meat.

The use of nitrite and nitrate in meat products and other foodstuffs is controlled both by local and international regulations. A review was therefore carried out of all relevant local regulations as well as the parallel provisions on the same matter in European and American legislation. Reference was also made to published international standards (in particular European standards and the Codex standards prepared by the Joint FAO/WHO Food Standards Programme) on processed meat.

The main focus of the entire project was certainly the survey of levels of nitrite and nitrate in 25 local and 25 imported meat products. The aim of the survey was to validate a standard analytical method and check whether levels of such preservatives in processed meat products currently available in the local market, correspond to normal levels found elsewhere and to compare them with values obtained by the last (unpublished) survey conducted by the local
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Public Health Laboratories (Public Health Labs, Malta, 1996). The survey also provided the opportunity to determine whether products analysed were within legal regulatory limits of particular additives under study.

1.5 Survey Action Plan

Twenty five locally processed and the same amount of imported meat products were randomly selected and analysed for nitrite and nitrate using a 'standard' published method. In general, methods available tend to show wide variations in techniques for extraction, clean-up of extracts, and final determination steps.

The methods chosen for this particular survey were based on the most frequently employed techniques (AOAC, 1995; ISO, 1975; BSI, 1976). Twelve determinations were carried out on each product, six for the measurement of nitrite level and another six for nitrate level, calculating mean values, standard deviations and standard errors in each case.

Samples were initially homogenised, extracted in hot borax solution and then analysed as follows:

(i) for nitrite, using a spectrophotometric assay following the formation of a diazonium salt with a primary aromatic amine in
acid solution, and coupling with an aromatic compound bearing
an influential amino substituent to form an azo pigment;

(ii) for nitrate, by reduction of nitrate present in the meat extract
by cadmium metal suspended in a glass column, followed by the
same spectrophotometric technique for nitrite determination,
at the end of the aforecited diazotisation-coupling reaction.

Although the method chosen was simple in principle, the
procedure turned out to be quite lengthy, requiring a total duration
of about 5 hours (excluding occasional troubleshooting) to completely
analyse one product by carrying out preparation/homogenisation of
sample, extraction and a whole set of nitrite/nitrate determinations.

The precision of the method employed could finally be
calculated on the basis of all the values obtained. Although the
overall recovery of nitrite and nitrate, added to fresh samples of beef
was calculated and found to be satisfactory, it must be said that the
reliability and hence accuracy of the method could only be
determined via a number of inter-laboratory trials.
2.1 Bacterial Spoilage of Meat and Means of Control

The meat of a healthy animal is sterile. However, bleeding during slaughter, introduces bacteria from the skin of the animal and the knife of the operator into the bloodstream. Such microorganisms are quickly carried throughout the tissues by the residual blood circulation. The situation is even worsened by the accidental invasion of the bloodstream by intestinal bacteria which could happen during slaughter.

Very often, meat will start getting externally contaminated from the environment in the abattoir. In spite of the most stringent precautions, the initial microbial count will increase sharply from the following sources (Lawrie, 1979):

(a) presence of animal skins (hides) with their adhering soil, lymphatic system and gastrointestinal contents;
(b) airborne microorganisms;
(c) use of knives, cleavers and saws;
(d) water used for cleaning carcasses;
(e) equipment;
(f) personnel.
The HACCP (Hazard Analysis Critical Control Point) practice, which aims to identify the relative seriousness of the various risks associated with each step of a process, will certainly control but not eliminate such sources of microbes. These sources frequently determine the range of microbial contaminants present and thus the potential for pathogenic hazard or product spoilage.

The HACCP concept in meat production is still important because it pinpoints systematically any potential hazards in the entire chain, from animal production to consumption, and ranks them according to severity and likely frequency of occurrence. It covers facilities, equipment and operation and is intended to augment and refine the various codes of manufacturing practice undertaken by industry (Bender, 1992).

Animal carcasses are known to support the growth of both aerobic and anaerobic organisms (bacteria, yeasts, moulds). Microbial growth depends on:

(i) the initial contamination (from various sources), and
(ii) storage conditions.

Usually, the effect is decomposition and putrefaction brought about by non-pathogenic bacteria.

Since storage conditions usually include low temperature (≤ 5°C), the main survivors are usually (Hawthorn, 1981):
i) psychrophilic bacteria (e.g. Achromobacter, Micrococi, Lactobacilli & Pseudomonas);

ii) moulds (e.g. Penicillium & Cladosporium species).

The main factors affecting growth are:

(i) temperature;
(ii) humidity;
(iii) pH.

The presence or absence of salts such as sodium chloride and sodium nitrite would influence both growth rates and the types of organisms developing, nitrite being an effective inhibitor of Cl. botulinium.

Some food poisoning organisms may also develop. These include Salmonella species, Cl. welchii, Staphylococci and Enterococci. Luckily enough, poisoning by Cl. botulinium, the most deadly of organisms, is quite rare compared to outbreaks associated with the other species.

Table 1 illustrates the mechanisms through which preservation techniques achieve their objectives in foods. Information in the table is generalized and not all microorganisms are essentially controlled by each mechanism. In general, low pH (4-5), low temperature (-29 to 40°C) and low humidity (< 90%) result in the maximum suppression of microbial activity in fresh sides of meat (Hawthorn, 1981). However such conditions may result in:
i) excessive drying of meat surfaces leading to loss of "bloom" (i.e. attractive appearance is lost);

ii) excessive weight loss by evaporation, implying reduced profits to the trader.

At higher temperature and humidities (e.g. 4°C and 90% relative humidity), the conditioning process of meat proceeds at a faster pace but may result in some growth of surface mould. In meat curing, a temperature of 4-6°C is used; in this case growth of halophilic organisms in the curing brine is deliberately encouraged since such organisms are able to reduce nitrate to nitrite. In fact, bacon is stored for 7-14 days at a temperature of 4-6°C to allow curing salts to diffuse evenly through the tissues. It is under such conditions that the characteristic bacon flavour develops.

Table 2 illustrates some of the control factors or mechanisms that operate in cured meats (and some smoked fish). The symbols indicate that a particular control mechanism operates on target organisms. Hence not all microorganisms occurring in the product are necessarily inhibited by a particular mechanism. Some factors are extremely effective in minimizing microbial growth. However, a combination of factors are usually employed to reduce proliferation of such organisms.
### Table 1

**Mechanisms Whereby Preservation Methods Control Microbial Proliferation In Foods**

*(Source: US Assembly of Life Sciences, 1982)*

<table>
<thead>
<tr>
<th>Predominant Effect on Microbial Contaminants</th>
<th>Cause</th>
<th>Examples of Preservation Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactivation or injury</td>
<td>Increased temperature, Radiation</td>
<td>Thermal processing, pasteurization, Radappertization (radiation)</td>
</tr>
<tr>
<td>Inhibited&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Low temperature, Low water activity, Low pH, Chemical Inhibition, Microbial competition, Gaseous atmosphere&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Refrigeration, freezing, Drying, salting, curing, sugar addition, Acidulation, some fermentations, Curing, salting, natural smoking, Fermentations, Vacuum packaging</td>
</tr>
</tbody>
</table>

<sup>a</sup>some of the causes of inhibition, e.g., salting, may also cause death of some microbial species.

<sup>b</sup>some methods act by more than one method or indirectly, e.g., fermentation can be viewed as microbial competition that lowers the pH or produces alcohol, which acts as a preservative.

<sup>c</sup>unfavourable redox potential may also inhibit microbial proliferation but is not directly manipulated in any current preservation method.
### Table 2

**Influences on Microbial Survival or Proliferation for Various Cured Red Meat, Poultry, and Fish Products**

*(Source: US Assembly of Life Sciences, 1982)*

<table>
<thead>
<tr>
<th>Heating</th>
<th>Inhibition of Undesirable Microbial Proliferation by</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inactivation</td>
</tr>
<tr>
<td>Raw, cured meat products</td>
<td></td>
</tr>
<tr>
<td>Bacon</td>
<td>+</td>
</tr>
<tr>
<td>Other pickle-cured products e.g. ham</td>
<td>+</td>
</tr>
<tr>
<td>Dry-cured cuts, e.g. country hams</td>
<td>0</td>
</tr>
<tr>
<td>Dry, semidry, &amp; fermented sausages</td>
<td>+</td>
</tr>
<tr>
<td>Cooked, cured meat products</td>
<td></td>
</tr>
<tr>
<td>Perishable (pasteurised), packaged after heating, e.g. frankfurters</td>
<td>+</td>
</tr>
<tr>
<td>Perishable (pasteurised, canned, e.g. ham</td>
<td>+</td>
</tr>
<tr>
<td>Shelf-stable, canned, e.g. luncheon meats</td>
<td>+</td>
</tr>
<tr>
<td>Commercially sterile, e.g. deviled ham</td>
<td>++</td>
</tr>
<tr>
<td>Smoked Fish</td>
<td>+</td>
</tr>
</tbody>
</table>

**Key:**
- Reduced effectiveness in controlling undesirable microbial proliferation.
- Not involved in controlling microbial proliferation.
- Supplementary control on microbial proliferation.
- Major control on microbial proliferation.

**Notes:**
- Some raw products are mildly heated. Vegetative cells of most bacterial species are inactivated by the temperatures used in heating bacon and other pickle-cured products, some fermented sausages, and cooked products. Inactivation of spores requires more severe heating as used for commercially sterile and shelf-stable products. Inactivation of spores requires more severe heating as used for commercially sterile and shelf-stable products. Commercial sterilisation results in a minimum of 12 log cycles reduction in the number of viable *C. botulinum* spores in products receiving a "botulinum" cook, but thermal processing of shelf-stable products yields only approximately a 3-log reduction. Remaining viable spores are damaged and, thus, are more sensitive to the effects of other controls.
- If recommended.
- NaCl and NaNO₂. Preservatives in commercially sterile products may inhibit microbial proliferation if inadequate thermal processing has allowed viable cells or spores to survive.
- Naturally occurring (or in fermented sausages, added) lactic-acid-producing bacteria, which are generally inactivated by cooking, may lower pH, if fermentable carbohydrate is present.
- Vacuum packaging of cured products controls aerobic spoilage organisms, but generally does not affect proliferation of *C. botulinum*. 
2.2 Technics of Processing & Preservation of Meat

Meat processing is no longer aimed exclusively as a method of preservation; it is now regarded also as a source of variety to the human diet by bringing about several desirable changes in texture and flavour to the product. Processing today aims also at:

(a) exploiting meat resources to the full by mixing the less desirable parts of the carcass with lean meat, and

(b) extending meat supplies by including other foodstuffs such as cereal in the product.

Meat is considered as a highly perishable product and would soon turn unfit and possibly unsafe through microbial growth, chemical change and enzymatic activity. Such processes may be controlled by employing one or more of the following measures (Lawrie, 1979):

- by reducing the temperature sufficiently to slow down or inhibit microbial growth;
- by heating to destroy organisms and enzymes (e.g. cooking, canning);
- by removal of water via drying or osmotic control (i.e. binding water with salt/other solutes, making it unavailable to the organisms);
- by adding antimicrobial agents to inhibit growth;
- by using ionising radiation (still not permitted in some countries).
Certain traditional methods of meat processing (such as drying in the wind/sun, salting and smoking) have been used for thousands of years. On the other hand, canning dates from early 19th century (Bender, 1992).

2.2.1 Chilling & Freezing

Mechanical refrigeration is regarded as a relatively modern technique of meat preservation. Meat is usually ‘chilled’ by storing it for several days at a temperature of -1°C to 4°C. Modern packaging techniques including storage under carbon dioxide or nitrogen or in vacuum can extend period of storage to about 10 weeks provided meat is kept very cool (-1°C to 0°C) and that strict hygienic procedures are followed at the slaughterhouse and factory.

Chilling (especially at temperatures close to freezing point of meat, i.e. -1.5°C) reduces risks associated with pathogenic bacteria and slows down growth of spoilage organisms. Although chilling inhibits most pathogens (e.g. Salmonella, Staphylococcus species and Clostridium perfringens), others may survive (e.g. Listeria monocytogenes at +2°C, some Salmonella species at +5°C, Campylobacter at +7°C). Spoilage organisms associated with meat include Pseudomonas species (which may be found on the exposed surface of chilled meat) and Lactobacilli (on vacuum-packed meat).
Domestic Freezing (at -18°C) and Commercial Freezing (at -29°C) are standard techniques which can be used to preserve meat products for periods of 1-2 years. However, long period of storage of frozen meat may lead to some deterioration (both microbial and organoleptic) of eating quality compared with fresh or chilled meat (Bender, 1992).

2.2.2 Processing

Processed meats are products in which the properties of fresh meat have been modified by the use of procedures such as mincing, grinding or chopping, salting and curing, addition of seasonings and other food materials, and very often heat treatment. In general, they may be considered as emulsions of protein, fat and water and hence their manufacture depends mostly on the ability to retain water (Bender, 1992).

2.2.2.1 The Curing Technique

Although the term 'curing' was originally applied to preservation in general, it is now restricted to preservation with salt (sodium chloride) and sodium or potassium nitrite or nitrate or a mixture of these two salts. The nitrate is believed to serve as a source for bacterial reduction to nitrite (Wilson, 1981).

Salt inhibits growth of most spoilage organisms at concentrations greater than 4%. It may function as a complete preservative when concentration is approximately 17%; however, at
these levels, the product is rendered practically unpalatable. So, in the case of cured meat products, salt concentration is kept as low as 2.5-5% with nitrite inhibiting the growth of other organisms (Bender, 1992). Addition of nitrite is beneficial because it is known to react with proteins when heated to form compounds (the Perigo-type factors) which are able to inhibit germination of spores of pathogenic bacterium *Clostridium botulinum*.

Apart from its antimicrobial function, nitrite contributes to the development of a desirable pink coloration and flavour associated with the curing process.

The curing operation which used to be a lengthy process, has been recently developed to take considerably less time. For example:
- meat can be cured in 1-2 weeks by being initially injected with the curing solution rather than being simply left immersed in brine;
- bacon, usually cured in a few hours, can be processed in a few minutes if heated, provided the cure is completed in the final package.

The curing process may also be accelerated by adding sodium ascorbate which acts as an additional reducing agent and
allows utilisation of small amounts of nitrite, thereby reducing possibilities of the formation of carcinogenic nitrosamines. The antioxidant properties of ascorbic acid assist in stabilising the colour and preventing rancidity problems. Roberts & Ingram (1977), however, question the complex effects of sodium ascorbate, polyphosphate and even sodium nitrate in the curing process.

2.2.2.2 Tumbling & Massaging

This is a new technique, developed in the 1960s, which accelerates penetration of salt by injecting the curing salt solution into pieces of meat or immersing chopped meat into the solution followed by mechanical shaking or “tumbling”.

Solutions of salt (2-8% concentration) are then added together with polyphosphate and part of the water-soluble protein (mainly myosin) is extracted. The process improves the water-holding capacity of the meat by reaction of salt with structural proteins, aided by the polyphosphate. The extracted proteins then set to a strong gel on heating and bind together the pieces of meat which can then be shaped or sliced.

If the mechanical treatment is not vigorous, the term “massaging” is used instead to describe this process (Bender, 1992).

2.2.2.3 Smoking
Smoking is another traditional technique of meat preservation. It improves the keeping quality of meat due to:

(a) desiccation at the surface;
(b) presence of bacteriostatic agents in the wood smoke which are deposited on the surface.

In fact, smoking introduces large amounts of antioxidants such as butylated hydroxyanisole (BHA) and butyl gallate. Permitted levels of such antioxidants (as additives) are far below levels found in smoked meat (Grierson, 1997).

The process has changed with time with meat being now treated with smoke produced from wood sawdust in a generator, rather than deriving from a common wood fire.

The deposit on meat left by smoking contributes also to the flavour and appearance of the product; however, if light smoking is applied, the preservative effect is limited and the product must be refrigerated.

If the product is subjected to intensive smoking, the shelf-life is prolonged by having more substances deposited on meat and by the drying effect of hot air. This may have a detrimental effect on the flavour and is therefore only applied when other methods are not possible.

New developments in smoking made it possible for meat
to be treated with an aqueous solution containing the main constituents of smoke.

2.2.3 Other Means of Preservation

Other effective methods of preservation of meat include:

I. **Drying** (including Freeze Drying) involving the removal of moisture from the outer layers of thin pieces of meat and the migration of moisture from the inside to the outside. Microorganisms cannot proliferate in the absence of sufficient moisture.

II. **Partial Drying (Intermediate-Moisture Foods)** by combining, an incomplete reduction in water activity (partial drying) with other measures such as lowering of pH, cooking or the addition of nitrate. Such products will keep for several months but may undergo changes in texture, colour and flavour.

III. **Canning** by sterilising (usually applying 'high temp-short time' - HTST - heating) the meat product in an air-tight can or bottle, or in a heat-resistant/aluminium foil-laminated plastic pouch. In the case of sausages, these are usually filled into retortable synthetic casings and sealed with aluminium clips.

IV. **Fat Embedding** by cooking the meat in a vessel which can be sealed under a layer of molten fat, thereby protecting it from
recontamination.

V. Ionising Radiation by subjecting the prepacked meat to ionising radiation produced from radioactive or electromagnetic sources. High doses (amounting to 50 kiloGrays) or irradiation are normally required for complete sterilization of meat. However, only doses up to about 10 kGy of radiation (enough to kill many pathogens) are usually allowed on food in many countries.

Recent developments in meat processing technology have also introduced on the market new products such as mechanically recovered meat (MRM), reformed meat products and products deriving from protein extraction (from by-products of low acceptability e.g. lungs, stomachs, etc.) (Wilson, 1981; Bender, 1992).

2.3 Potential Pathogens occurring in Cured Meats

Botulism is generally the most feared of foodborne diseases because of its traditionally high fatality rate, although statistics show that the rate has been decreasing in recent years. However, *Cl. botulinium* is not the only pathogenic organism that might occur in meat. Table 3 shows the frequency of disease outbreaks from various pathogens in meat and poultry products in USA over a period of 10 years (1968-77).
Table 3

Number of Meat and Poultry Products that were Reported as Foodborne Vehicles in Disease Outbreaks in the United States, 1968-1977

(Source: Bryan, 1980)

<table>
<thead>
<tr>
<th>Disease</th>
<th>Beef</th>
<th>Pork</th>
<th>Other meats</th>
<th>Meat, general</th>
<th>Poultry</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arizonosis</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Bacillus cereus</em> gastroenteritis</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Botulism</td>
<td>9</td>
<td>11</td>
<td>14</td>
<td>11</td>
<td>10</td>
<td>29</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em> gastroenteritis</td>
<td>4</td>
<td>13</td>
<td>22</td>
<td>8</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>9</td>
<td>4</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Shigellosis</td>
<td>9</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Staphylococcal intoxication</td>
<td>23</td>
<td>105</td>
<td>7</td>
<td>5</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Staphylococcal group D gastroenteritis</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>24</td>
<td>44</td>
<td>85</td>
<td>81</td>
<td>105</td>
<td>3</td>
</tr>
<tr>
<td>Toxoplasmosis</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Trichinosis</td>
<td>24</td>
<td>44</td>
<td>85</td>
<td>81</td>
<td>105</td>
<td>3</td>
</tr>
<tr>
<td>Chemical poisonings</td>
<td>24</td>
<td>44</td>
<td>85</td>
<td>81</td>
<td>105</td>
<td>3</td>
</tr>
<tr>
<td>Diseases of unknown etiology</td>
<td>47</td>
<td>73</td>
<td>153</td>
<td>123</td>
<td>226</td>
<td>408</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>408</td>
</tr>
<tr>
<td>Percent</td>
<td>3.4</td>
<td>5.2</td>
<td>11.0</td>
<td>5.8</td>
<td>1.3</td>
<td>5.3</td>
</tr>
</tbody>
</table>

(To be continued)
It should be noted that out of a total 15 outbreaks (39 cases, 16 deaths) of botulism, related to commercially processed meat or poultry, that occurred in USA or Canada over a period of 80 years (1899-1980), nearly 50% - 7 outbreaks (21 cases, 6 deaths) were attributed to “cured” products.

Table 4 shows the origins of the pathogenic organisms that occur in cured meats with information on the factors that may control their growth.

2.4 Risk of Botulism

There are various factors which can determine the potential risk for botulism. These include:

1) The presence of spores in the finished product due to inadequate heating which could be:
   (a) deliberate, i.e. intentional mild heating, or
   (b) accidental, i.e. due to faulty thermal processing.
   This may result in suppression of competitive organisms.

2) The influence of the intrinsic characteristics of the finished product (e.g. pH, concentration of brine) on the control Cl. botulinium spore outgrowth and cell multiplication.
### Table 4
Characteristics of Potential Pathogens Occurring in Cured Meats
(Source: US Assembly of Life Sciences, 1982)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Clostridium botulinum</th>
<th>Clostridium botulinum</th>
<th>Staphylococcus aureus</th>
<th>Salmonellae</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group I</strong>, proteolytic types A &amp; B</td>
<td><strong>Group II</strong>, non-proteolytic types A, B &amp; F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Reservoir</strong></td>
<td>Soil</td>
<td>Soil, sediments, water</td>
<td>Livestock, man</td>
<td>Animals, including man</td>
</tr>
<tr>
<td><strong>Vehicle of Transmission</strong></td>
<td>Soil-derived dust, non-meat ingredients</td>
<td>Dust, water</td>
<td>Nose, skin, lesions</td>
<td>Contact with faeces</td>
</tr>
<tr>
<td><strong>Transmission</strong></td>
<td>As spores</td>
<td>As spores</td>
<td>As vegetative cells</td>
<td>As vegetative cells</td>
</tr>
<tr>
<td><strong>Disease Agent</strong></td>
<td>Neurotoxin</td>
<td>Neurotoxin</td>
<td>Enterotoxin</td>
<td>Vegetative cells, multiplying in gastrointestinal tract</td>
</tr>
<tr>
<td><strong>Time, Temp. Requirements for Production of Toxic or Infective Levels</strong></td>
<td>Days at 10-50°C (optimum, 37°C)</td>
<td>Days at 3.3-50°C (optimum, 30°C)</td>
<td>5 hours at 7.8-43°C (optimum, 37°C)</td>
<td>Zero to several hours at 6.7-45.6°C (optimum, 37°C)</td>
</tr>
<tr>
<td><strong>Growth Requirements</strong></td>
<td>Anaerobic</td>
<td>Anaerobic</td>
<td>Aerobic, facultatively anaerobic</td>
<td>Aerobic, facultatively anaerobic</td>
</tr>
<tr>
<td><strong>Heat Resistance:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Organism</strong></td>
<td>High (spores)</td>
<td>Moderate (sp.)</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td><strong>Toxin</strong></td>
<td>Low</td>
<td>Low</td>
<td>High</td>
<td>NA&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Limiting Conditions&lt;sup&gt;b&lt;/sup&gt; for Proliferation or Toxin Production:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Temp, °C</strong></td>
<td>&lt; 10</td>
<td>&lt; 3.3</td>
<td>&lt; 7.8</td>
<td>&lt; 6.7</td>
</tr>
<tr>
<td><strong>Water Activity</strong></td>
<td>&lt; 0.94</td>
<td>&lt; 0.95</td>
<td>&lt; 0.87</td>
<td>&lt; 0.95</td>
</tr>
<tr>
<td><strong>Brine, %</strong></td>
<td>&gt; 8-10</td>
<td>&gt; 5</td>
<td>&gt; 12-13</td>
<td>≈ 5.3</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>5.5-5.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.5-5.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.7</td>
<td>4.5</td>
</tr>
<tr>
<td><strong>Effect of:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Microbial Competition</strong></td>
<td>Very inhibitory, especially at low pH</td>
<td>Very inhibitory, especially at low pH</td>
<td>Very inhibitory, especially at low pH</td>
<td>Inhibitory, especially lactic acid bacteria</td>
</tr>
<tr>
<td><strong>Sodium Nitrite (120-200 mg/kg)</strong></td>
<td>Effective</td>
<td>Effective</td>
<td>May act anaerobically, not effective</td>
<td>Not effective</td>
</tr>
</tbody>
</table>

---

<sup>a</sup> *Clostridium perfringens* may be present, but is not a major cause of disease outbreaks involving cured meats.

<sup>b</sup> These values may differ if other growth conditions are sub-optimal. Values are for growth in meat products, where data were available.

<sup>c</sup> Value is typical for cured meats, which contain salt.

<sup>d</sup> NA = not applicable.
3) The exposure of product to ideal growth conditions such as:

- exposure to ambient temperature of shelf-stable products (e.g. canned luncheon meats) and commercially sterile products (e.g. deviled ham, Vienna sausages) that may have been inadequately thermally processed;
- exposure to high temperature of perishable products (e.g. ham).

Figure 1 summarises the sequence of events leading to botulism.

The risk of botulism in meat products can certainly be reduced through the addition of nitrite or an alternative antimicrobial agent. However, protection against toxigenesis from *Cl. botulinum* spores (which are so ubiquitous) can also be achieved by controlling factors such as:

i) *degree and frequency of microbial contamination (which can occur during slaughter, processing, and/or handling procedures).*

Spore contamination of the finished product depends on initial contamination and the severity of thermal processing.
Spores in soil

Dust

Animal faeces
Equiment
Workers

Carcasses during slaughter & processing

Equipment

Storage

Transportation

Storage

Equipment

Meat during cooking

Workers

Cooking
Spores survive.
Spores are heat-shocked.
Competitive organisms are killed.
Food becomes more anaerobic.

Storage
Time-temperature conditions during storage allow spore germination, vegetative cell multiplication, and toxin production.

Inadequate reheating to destroy toxin.

Ingestion

Figure 1
Web of causation of *Clostridium botulinum* food-borne intoxication.
(Modified from Bryan, 1979)
For fish, the reservoir of spores is water or sediments.
i) *multiplication during production, distribution, storage and handling.* Pathogenic organisms may proliferate during the production of cured products and at a later stage.

Table 5 summarises the various opportunities given to pathogens to grow and increase in count.

ii) *intrinsic characteristics of product other than nitrite.* The extent to which product characteristics such as pH and water activity is considered to be a critical factor in evaluating the risk from cured products (especially, if depleted of any chemical preservatives).

Table 6 summarises the characteristics of US cured products that may influence microbial growth. Proliferation is thought to be controlled by the interactions of the various factors which may be additive, synergistic or antagonistic.

Studies on the relative susceptibilities of various classes of products to toxigenesis revealed that pasteurised products, especially those with low salt concentrations and those made from poultry, are at greatest risk under conditions of temperature abuse. Other products seem to have greater resistance to toxigenesis e.g. fermented sausage and some types of bacon have other factors (such as high salt concentration) that may limit pathogenic proliferation. However, the addition of an antimicrobial preservative such as nitrite is still regarded as essential for the ultimate safety of the product (US Assembly of Life Sciences, 1982).
Table 5
Influence of Production, Storage, Distribution, and Handling on Pathogenic Riska
(Source: US Assembly of Life Sciences, 1982)

<table>
<thead>
<tr>
<th>Opportunities for Increased Contamination of Product Presented During</th>
<th>Opportunities Presented for Microbial Growth During</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processing</td>
<td>Packaging</td>
</tr>
<tr>
<td>Raw, cured products</td>
<td></td>
</tr>
<tr>
<td>• Bacon</td>
<td>NI</td>
</tr>
<tr>
<td>• Other pickle-cured products, e.g., hams</td>
<td>NI</td>
</tr>
<tr>
<td>• Dry-cured cuts, e.g., country hams</td>
<td>NI</td>
</tr>
<tr>
<td>• Dry, semidry, and fermented sausages</td>
<td>NI</td>
</tr>
<tr>
<td>Cooked, cured products</td>
<td></td>
</tr>
<tr>
<td>• Perishable (pasteurised), packaged after heating, e.g., frankfurters</td>
<td>From equipment surfaces during comminution</td>
</tr>
<tr>
<td>• Perishable (pasteurised), canned, e.g., hams</td>
<td>NI</td>
</tr>
<tr>
<td>• Shelf-stable, canned, e.g., luncheon meats</td>
<td>From equipment surfaces during comminution</td>
</tr>
<tr>
<td>• Commercially sterile, e.g., deviled ham</td>
<td>From equipment surfaces during comminution</td>
</tr>
</tbody>
</table>

a This table focuses on the risk from Clostridium botulinum, but similar (although not identical) considerations apply to Staphylococcus aureus and salmonellae.

b Sufficient cooking may inactivate botulinum toxin, but no staphylococcal enterotoxin. Some heating, e.g., during use of bacon as a flavouring agent, may not be adequate to destroy botulinum toxin. Many products are only very rarely, if ever, cooked.

c NI = no notable influence.

d Growth of staphylococci is the major problem under these circumstances.
### Table 6

**Intrinsic Characteristics of Cured Products other than Preservatives** \(^a\) **that may minimize the Proliferation of Pathogens** \(^b,c\)  
(*Source: US Assembly of Life Sciences, 1982*)

<table>
<thead>
<tr>
<th>Raw, cured products</th>
<th>Water Activity</th>
<th>Brine, %</th>
<th>pH</th>
<th>Microbial Competition (^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacon</td>
<td>&gt;0.95</td>
<td>3.0-6.0</td>
<td>≈(5.6-6.4)</td>
<td>Possible</td>
</tr>
<tr>
<td>Other pickle-cured products, e.g. hams</td>
<td>&gt;0.95</td>
<td>3.0-6.0</td>
<td>≈(5.6-6.4)</td>
<td>Possible</td>
</tr>
<tr>
<td>Dry-cured cuts, e.g. country hams</td>
<td>≈0.92</td>
<td>Increases during production to ≈ 10 (internal)</td>
<td>≈(5.6-6.4)</td>
<td>Possible(^f)</td>
</tr>
</tbody>
</table>

- **Dry, semidry, and fermented sausages:**
  - Cooked: ≈0.95, ≈(10-16), ≈(4.8-5.2) Possible\(^e\)
  - Semidried: <0.92, ≈(10-16), ≈(4.8-5.2) Encouraged\(^f\)
  - Dried: <0.92, ≈(10-16), ≈(4.8-5.2) Encouraged\(^f\)

**Cooked, cured products**

- Perishable (pasteurised), packaged after heating, e.g. frankfurters: >0.95, 3.5-5.5, ≈(5.6-6.4) Possible
- Perishable (pasteurised), canned, e.g. hams: >0.95, 3.5-5.5, ≈(5.6-6.4) Unlikely (minimized)
- Shelf-stable, canned, e.g. canned luncheon meats: >0.95, 3.5-5.5, ≈(5.6-6.4) Unlikely (minimized)
- Commercially sterile, e.g., deviled hams: >0.95, 3.5-5.5, ≈(5.6-6.4) Unlikely (minimized)

- Sodium chloride and sodium nitrite. 
- Potential pathogens of concern are *Clostridium botulinum*, *Staphylococcus aureus* and salmonellae.
- A number of other intrinsic factors undoubtedly affect microbial proliferation, e.g., meat species, type and cut, and curing adjuncts (such as phosphates and ascorbate), but much less is known about their influence.
- From natural flora if fermentable carbohydrate added.
- By back-inoculation or with starter culture.
- Because recontamination may occur during packaging.

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\(^a\) Potential pathogens of concern are *Clostridium botulinum*, *Staphylococcus aureus* and salmonellae.

\(^b\) A number of other intrinsic factors undoubtedly affect microbial proliferation, e.g., meat species, type and cut, and curing adjuncts (such as phosphates and ascorbate), but much less is known about their influence.

\(^c\) From natural flora if fermentable carbohydrate added.

\(^d\) By back-inoculation or with starter culture.

\(^e\) Because recontamination may occur during packaging.
2.5 **Product Susceptibility to Spoilage**

Spoilage (second aspect of preservation) involves a wider range of microorganisms than those producing a health hazard. Apart from being so different and so numerous among products, less is known about the specific organisms involved (e.g. factors affecting growth, contributions of the various factors controlling growth, etc.). Table 7 illustrates the relationship between spoilage problems in cured meats and the type of product.

The factors determining product spoilage are basically similar to those which determine the possibility of pathogenic hazard, that is:

1. Extent of initial microbial contamination of product and effects of processing e.g. thermal processing.
2. Capacity of product itself to control growth of spoilage organisms (via intrinsic characteristics).
3. Exposure of product to temperatures that allow microbial growth.

In the absence of antimicrobial agents such as nitrite, spoilage organisms such as anaerobic and aerobic spore-forming bacteria, which promote putrefaction (e.g. clostridia and bacilli) may become a problem when products are exposed to temperatures allowing their growth. Such spores (which outnumber those of Cl...
### Table 7

**Microbial Defects of Red Meats and Their Products**

*(Source: Banwart, 1979)*

<table>
<thead>
<tr>
<th>Product</th>
<th>Defect</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fresh meat</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Fresh, refrigerated (0-5°C)</td>
<td>Off-odour, slime, discolouration</td>
<td>Pseudomonas, Aeromonas, Alcaligenes, Acinetobacter, Microbacterium, Moraxella, Proteus, Flavobacterium, Alteromonas, Saccharomyces.</td>
</tr>
<tr>
<td>• Fresh (15-30°C)</td>
<td>Bone taint</td>
<td>Clostridium</td>
</tr>
<tr>
<td>• Vacuum-packaged</td>
<td>Acid, sweet, rancid</td>
<td>Lactobacillus, Microbacterium, Enterobacter, Hafnia.</td>
</tr>
<tr>
<td><strong>Cured meat</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Bacon (vacuum-packaged)</td>
<td>Cheesy, sour, rancid</td>
<td>Micrococcus.</td>
</tr>
<tr>
<td>• Other vacuum-packaged meats</td>
<td>Cabbage odour</td>
<td>Proteus inconstans.</td>
</tr>
<tr>
<td>• Brines</td>
<td>Tainted</td>
<td>Vibrio.</td>
</tr>
<tr>
<td>• Ham</td>
<td>Surface slime</td>
<td>Micrococcus, Microbacterium, yeasts.</td>
</tr>
<tr>
<td>• Sausages</td>
<td>Gassy or puffy</td>
<td>Clostridium.</td>
</tr>
<tr>
<td>• Sausages</td>
<td>Green discoloration</td>
<td>Lactobacillus, Streptococcus, Leuconostoc.</td>
</tr>
<tr>
<td>• Fermented sausages</td>
<td>Surface slime</td>
<td>Micrococcus, yeasts.</td>
</tr>
<tr>
<td>• Fermented sausages</td>
<td>Gas production (vacuum-packaged)</td>
<td><em>Lactobacillus viridescens</em>, Leuconostoc.</td>
</tr>
<tr>
<td>• Fermented sausages</td>
<td>Greenish discoloration</td>
<td></td>
</tr>
<tr>
<td><strong>Canned Meat</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Commercially sterile</td>
<td>Gas, putrefaction</td>
<td>Spore-formers (Bacillus, Clostridium).</td>
</tr>
<tr>
<td>• Semi-preserved</td>
<td>Souring, discolouration</td>
<td>Streptococcus.</td>
</tr>
<tr>
<td>• Semi-preserved</td>
<td>Putrefaction, gas</td>
<td>Bacillus, Clostridium.</td>
</tr>
</tbody>
</table>
botulinium, according to Holley, 1978) may be more resistant to the lethal effects of heat and other agents (International Commission on Microbiological Specifications for Foods, 1980).

Hence, it was determined that the products that are most likely to spoil (in the absence of an antimicrobial agent) are:

1) **Shelf-stable products**

which are given less than a botulinium cook (i.e. heating at 120°C for 4 minutes) and hence do not receive thermal processing adequate to kill spores of putrefactive spore-forming bacteria, and which are stored at temperatures allowing spore outgrowth;

2) **Perishable Canned Products**

(e.g. ham) because such products are more often subjected to temperature abuse, such as unrefrigerated storage, at retail or by the consumer.

Addition of the right amount of antimicrobials (e.g. nitrite) is also important to inhibit the growth of some psychrotrophs, under refrigerated conditions (Terrell, 1974).

It should be stressed that spoilage should not pose a major problem if careful attention is paid to:
Chapter 2

PRESERVATION OF MEAT PRODUCTS

* hygiene and equipment sanitation during production and packaging of products, and
* continuous and correct refrigeration.

However, these involve considerable uncertainties that arise from the possibility of human error or mechanical failure.

2.6 Interrelationship of Spoilage & Pathogenic Organisms

When one is considering means of control of microbial growth in meat products, one should also bear in mind the interrelationship between spoilage and health hazard. For example, some spoilage microorganisms such as lactobacilli, may exert an inhibitory effect on growth of pathogenic organisms through microbial competition or other means such as the production of acid from fermentable carbohydrate added to the product.

Having said that however, one has to take also into account that products may become toxic before they are spoiled. So, spoilage cannot itself be envisaged as a means of reducing risks from pathogens because it may eventually end up in rendering a product which is still not fit for human consumption.
2.7 Interaction of Factors controlling Pathogens & Spoilage Organisms

The need of antimicrobial agents in cured meat products may be reduced if one were to optimise the intrinsic factors that exert control over microbial proliferation.

Such variables which need special attention are:

a) **Product composition**
   - including cut of meat used and the species from which it was taken;

b) **Product characteristics**
   - e.g. pH, $a_w$, $E_h$ and brine concentration;

c) **Types and concentrations of curing adjuncts**
   - e.g. phosphates, ascorbates, antioxidants, chelates and nitrosation inhibitors;

d) **Processing conditions**
   - e.g. comminution (which may affect product uniformity) and temperature and duration of heat treatment;

e) **Atmosphere in which a product is enclosed**;

f) **Incubation temperature**
   - i.e. simulated abuse of temperature;

g) **Storage conditions**
   - subjecting product to various temperatures for different periods before temperature abuse;
h) Degree of contamination

- e.g. spore load.

The Committee on Nitrite & Alternative Curing Agents in Food, set up by the US Assembly of Life Sciences (1982) reports that “high priority should be accorded to investigations of the interaction of (such) factors controlling pathogens and spoilage organisms in different commercial products in order to develop methods for predicting the degree of control gained or lost through alteration of any of these factors”.

The same committee suggests periodic evaluation of the need of antimicrobial preservatives (such as nitrite) in various products or product types since:

◊ new information may become available, and
◊ processing procedures may change with time.
3.1 Choosing the Right Antimicrobial Preservative

Only about 30 different compounds (out of a list running in hundreds of permitted food additives) can be legally used as "antimicrobials" in food products (Fulton, 1981). The choice of the right antimicrobial preservative depends on a number of factors which include:

3.1.1 Physical, Chemical & Antimicrobial Properties of Preservatives

Selection has to be based on the antimicrobial spectrum covered by the chemical in question. There are only a few chemicals that have the ability to inhibit several different types of microorganisms (broad spectrum). The antimicrobial spectrum of a compound may be determined by following the growth of the organisms in the presence of various concentrations of the antimicrobial.

Antimicrobials preserve food in one of two ways:

i. Bacteriostasis: by controlling the overall growth of
microorganisms, or

ii. **Lethality:** by directly destroying all or part of the microorganisms.

It is the mode of action of the chemical that determines whether bacteriostasis or lethality takes place.

The mode of action of an antimicrobial agent normally falls under one of 3 categories (Davidson & Branen, 1981):

1) reaction with cell membrane, causing increased permeability and loss of cellular constituents;

2) inactivation of essential enzymes;

3) destruction or functional inactivation of genetic material.

The overall microbial spectra, mode of action and efficiency of compounds are largely dependent on the chemical and physical properties of the antimicrobial (Davidson & Branen, Eds., 1991). Such properties include:

i) polarity of the compound;

ii) water solubility or hydrophilic properties (Robach, 1980);

iii) lipophilic properties (to some extent, if, say antimicrobial is to react with membrane of microorganism) (Branen et al., 1980);

iv) ability of compound to ionise;

v) chemical reactivity with other components of the food system (which should not, in any way, change the colour, flavour or texture of the food product).
3.1.2 **Properties & Composition of Food Product**

Antimicrobial activity can be lost if the antimicrobial reacts with the food components, resulting in:

- binding of the chemical to the food component, or:
- a breakdown/alteration of chemical structure of the antimicrobial.

In fact, several antimicrobials can ionise (at a certain pH), others can be oxidised or hydrolysed. Binding of antimicrobial (e.g. to proteins or lipids) probably results in the greatest loss of activity (Davidson & Branen, Eds., 1991).

Besides this, naturally occurring compounds (other than the major food components) can also influence the activity of the antimicrobial. Such compounds can in fact have a synergistic or antagonistic effect on the antimicrobial used.

3.1.3 **Characteristics, Level & Type of Microorganisms**

An antimicrobial is *never* a substitute for good sanitation in a food-processing plant and it is always desirable to have as low a microbial load as possible (Davidson & Branen, Eds., 1991). The choice of the antimicrobial, therefore, depends also on any treatment received by the product prior to the addition of the preservative in question.
If contamination is considerably high, significantly higher doses of antimicrobial have to be employed in the product. One must also be cautious not to select an antimicrobial solely according to the target organism because this may create favourable conditions for growth of other organisms.

3.1.4 Influence of Other Preservation Methods

One has to take also into account the type of preservation technique employed (in conjunction with the addition of the antimicrobial) since this would determine the total count and type of organisms present. For example:

i) heat processing would not eliminate all spore formers;

ii) low water activity would tend to promote those organisms (e.g. moulds/yeasts) which either survive or grow under such condition;

iii) refrigeration procedures generally favour psychrotrophic Gram-negative organisms;

iv) packaging resulting in low oxygen tension would favour proliferation of anaerobes.

3.1.5 Storage Conditions of Food Products

Storage temperature and time may also influence the efficacy of antimicrobials. They may, for instance, volatilise or react directly with other food components. In the long run (after extended
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storage time) they may even get metabolised by the microbial flora and be rendered ineffective.

3.1.6 Safety of Antimicrobial

The antimicrobial must definitely be safe for use in a food product. The safety of several antimicrobials has been questioned over the last decades and some have even been banned or limited to specific uses. In the meantime, attempts have been made to produce additive-free goods; however, it seems to be rather difficult to succeed in the current marketing systems with such products subjected solely to alternative preservation techniques.

An antimicrobial additive will only be considered safe if:

a) it proves to be non-toxic to test animals and humans;

b) it is metabolised and excreted by the body;

c) it does not result in the accumulation of residues in the body tissues upon reaction or decomposition.

The stringent legal requirements for safety, the length of the process of verification of safety and relevant costs (which, according to Robach, 1980, may reach well over $1 million before the official approval of a new additive) involved will definitely limit the drive to develop new antimicrobials. Yet, an important development seems to be in the offing with possible future trends to use naturally occurring antimicrobials such as nisin (and other compounds present
in the microorganism or in the product itself). Having already been in
the food supply and consumed for several years, such compounds
appear to be safe and may not require to undergo the level of testing
as in the case of new synthetic compounds. However, once isolated
and placed in other foods, it is uncertain whether such ‘natural’
antimicrobials would be safer than other compounds, i.e. natural
existence does not necessarily imply safety (Branden & Davidson, eds,

In spite of the parameters concerned, the assessment of
the overall safety of a particular antimicrobial has to take into account
the ability of the preservative to prevent the formation of lethal toxins
such as the botulinium toxin or carcinogenic aflatoxins.

3.1.7 Cost Effectiveness of Antimicrobial

One important criterion to be considered will certainly be
its cost effectiveness; in other words whether the chemical added
would truly reduce spoilage and minimize health hazards (foodborne
diseases) and justify its additional cost on the product.

In many cases, an additional 2 or 3 days of shelf-life can
significantly help to offset the cost of using a particular antimicrobial
additive. Hence, the efficiency of such preservatives is important in
determining an economical justification of their use in food products
(Branen, 1991).
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3.2  Microbiological Reasons for the Use of Nitrate & Nitrite

3.2.1  Historical Background

There is a long (and very often controversial) history of research on the possible antimicrobial properties and toxicology of nitrites, nitrates and their metabolites/residues.

Although early research work of the 1920s and 1930s focused on using nitrite to cure meat thereby aiming to tackle the problem of sour ham and temperature abuse of perishable canned hams in the marketplace, the indications till the early 40s were that nitrite was only involved in the development or restoration of flavour and colour in meat. On the other hand, nitrate was believed to provide the antimicrobial effect (Tompkin, 1980).

Further work conducted during 1940s and 1950s started proving the opposite. In fact, by the mid 50s, research had established that:

i. nitrite has significant antimicrobial properties;

ii. nitrous acid derived from nitrite in the acid conditions of meat, is responsible for a certain amount of microbial inhibition;

iii. nitrate served merely as a reservoir for the generation of nitrite.
Year 1969 and the early 70s were marked by the controversy on nitrosamines and pressure mounted throughout the decade to reduce or eliminate nitrate and/or nitrite form cured meat. By this time, the value of nitrate as a preservative had been so downgraded that it was generally agreed (at least in the United States) that nitrate could be omitted from cured meats without any effect on the antimicrobial protection/preservation of the product (Tompkin, 1980).

While there is no doubt about the antibotulinal properties of nitrite, the value of nitrate as a potential reservoir for conversion to nitrite and thus to nitrous acid is still under discussion while research continues to reveal new interactions among the chemical constituents of cured meat.

3.2.2 Clostridium botulinium

*Clostridium botulinium* is an anaerobic Gram-positive sporing soil bacterium that produces a potent neurotoxin (blocking neuromuscular transmission). The spores are heat resistant and can survive in foods that are incorrectly or minimally processed (e.g. food that is preserved or smoked but not cooked to 100°C).

Strains of *Cl. botulinium* are divided into 7 types (A-G) on the basis of their production of antigenically specific neurotoxins
(Sugiyama, 1980), and into 4 groups (I-IV), based on their proteolytic ability and other characteristics (Smith, 1977).

The organism and its spores are widely distributed in nature. They occur in both cultivated and forest soils, bottom sediments of streams, lakes and coastal waters and in the intestinal tracts of fish and mammals, and in the gills and viscera of crabs and other shellfish (US Food & Drug Administration, 1992).

*C. botulinum* is the organism responsible for "botulism" which is a rare but fatal illness brought about by the ingestion of the toxin produced by the bacterium. Outbreaks of botulism in humans are generally caused by strains of types A, B, E, or occasionally F, in groups I and II (US Assembly of Life Sciences, 1981). There are 2 main reasons why an outbreak of such food poisoning causes serious concern:

1) **high fatality rate**

- (about 31% according to Hawthorn, 1981, and over 50% according to Clegg & Clegg, 1987) The rate is very much higher than that associated with any other form of food poisoning. The US FDA reports 10-30 outbreaks of botulism a year in the USA. Some cases of botulism may go undiagnosed because symptoms are transient or mild, or misdiagnosed as Guillain-Barre syndrome (US Food & Drug Administration, 1992). Figure 2 shows
a graphical representation of reported cases of foodborne botulism in the US over the period 1988-1995.

![Graphical representation of reported cases of foodborne botulism, United States 1988-1995.](image)

(Source: Centers for Disease Control & Prevention, 1996)

1) unusual heat resistance of spores

- spores are completely destroyed by heating for 330 min at 100°C in phosphate buffer at pH 7, or by heating for 4 min at 120°C.

Being an anaerobe, the organism can grow and produce toxin in under-processed canned or bottled good. However it does not develop below pH 4.5. Low-acid foods (pH ≈ 4.5) are usually sterilised with a temperature-time combination that would ensure complete destruction of *Clostridium botulinum* spores.

The thermal process utilised to ensure safety and stability of low-acid canned foods was designed to destroy $10^{11}$ heat resistant spores of *Clostridium botulinum*, thus giving rise to the classic 12D concept,
where a D value implies 90% destruction of microbial count (Lechowich et al. 1978). In order to accomplish this 12D process in low-acid foods, drastic heat treatments (e.g. 2.78 min. equivalent at 250°F or higher) are required. However, many food products, especially canned cured meats, would be found organoleptically unacceptable after such heat treatments. Therefore commercial processing of these products involve heating for 0.05 to 0.6 min equivalent at 250°F, far below that of low-acid canned foods.

3.2.3 Types of Botulism

There are seven types (A, B, C, D, E, F and G) of botulism, depending on the antigenic specificity of toxin produced by each strain. While types A, B, E and F are usually associated with human botulism, types C and D cause most cases of botulism in animals (e.g. wild fowl, poultry, cattle, horses and some species of fish). Type G has been isolated from soil in Argentina; however, no outbreaks have yet been associated with such serotype. Toxins released by the various types of *Clostridium botulinum* are readily destroyed by cooking but this is not the case with their parent spores.

Botulism can also be classified under 4 categories:

1) foodborne botulism;
2) infant botulism;
3) wound botulism;
4) a form yet undetermined.

A simpler classification divides the disease in two: adult and infant types.

- **FOODBORNE BOTULISM** (treated in more detail under sections 3.2.4, 3.2.5, 3.2.6) is a foodborne intoxication caused by the consumption of foods containing the neurotoxin produced by *Clostridium botulinum*. It is usually associated with inadequately processed home-canned foods, but may also arise from commercially produced foods. The most frequent vehicles of foodborne botulism are sausages, meat products, canned vegetables and seafood products (US Food & Drug Administration, 1992).

- **INFANT BOTULISM** which was first recognised in 1976, affects infants under 12 months of age and is usually caused by the ingestion of *Clostridium botulinum* spores which colonize and produce toxin in the intestinal tract of infants (intestinal toxaemia botulism) (Centers for Disease Control & Prevention, 1995). Laboratory and epidemiological studies link infant botulism with honey, which serves as a potential dietary reservoir of *Clostridium botulinum* spores. To help prevent infant botulism, infants less than 12 months old should not be fed honey. Treatment for infants requires hospitalisation and possibly care in an intensive care unit. However, antitoxin is not recommended (Clayman, Ed,
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1994).

• **WOUND BOTULISM** is the rarest form of botulism. The illness results when *Cl. botulinium* by itself or with other microorganisms, infects a wound and produces toxins which reach other parts of the body via the blood stream. Such type of botulism does not involve foods (US Food & Drug Administration, 1992).

• The **UNDETERMINED CATEGORY** of botulism involves adult cases in which a specific food or wound source cannot be identified. Reports in medical literature suggest the existence of a form of botulism similar to infant botulism, but occurring in adults. Such disease might result from intestinal colonisation in adults, with in vivo production of toxin and may occur in patients undergoing alterations of the gastrointestinal tract and/or antibiotic therapy resulting in a change in normal gut flora (US Food & Drug Administration, 1992; Clayman, Ed., 1994).

3.2.4  **Foodborne Botulism**

Foodborne botulism is considered as a severe type of food poisoning caused by the ingestion of foods containing the potent neurotoxin formed during the growth of the organism. The toxin is heat labile and can be destroyed if heated at 80°C for 10 mins or longer (with spores, however, surviving such conditions) (US Food &
Foods involved in botulism may vary according to food preservation and eating habits in different regions. Botulism can be associated with any food that:

* is conducive to spore outgrowth and toxin production;
* allows spore survival, when processed, and
* is not subsequently heated before consumption.

Almost any type of food that is not very acidic (pH ≥ 4.6) can support growth and toxin production by *Clostridium botulinum*.

Botulinal toxin has been found in a large variety of foods such as canned corn, peppers, green beans, soups, beets, asparagus, mushrooms, ripe olives, spinach, tuna fish, chicken, chicken livers, liver pate, luncheon meats, ham, sausage, stuffed eggplant, lobster and smoked/salted fish.

The following are usually the conditions leading to foodborne botulism poisoning:

1. Food must be contaminated with *Clostridium botulinum* spores. The organism is so widely distributed in nature that it can be assumed that ALL raw food is contaminated (and thereby carries spores).
2. Food is treated in such a way that normal contaminating microorganisms are destroyed, leaving only spore-formers to survive with no competition. This is usually achieved in food subjected to mild heating, salting and pickling.

3. Composition of food is rendered suitable for growth or organisms and hence toxin production. Hence food must not be acidic (pH higher than 4.5).

4. Food must also be held at a suitable temperature for a sufficient time to allow growth and toxin formation.

5. Food is not cooked prior to consumption. boiling, even for a few minutes, is sufficient to inactivate the toxin.

6. Being an obligatory anaerobe, Cl. botulinium can only survive and proliferate in the absence of air. Such anaerobic conditions can be provided by certain food processing techniques namely canning, potting, pickling and smoking. Foodstuffs that are vacuum packed or contained in hermetically sealed cans/bottles and the interior of a smoked ham or fish are considered to be in anaerobic conditions.

There are several types of foodstuffs which may be exposed to such conditions. These include:
i) under-processed (or uncooked) home-bottle vegetables;
ii) lightly salted cured meats;
iii) fish pickled without enough acid;
iv) lightly salted, smoked and partly dried fish.

Other factors which are likely to determine whether a toxic product will be eaten or whether it will cause botulism if ingested include:

- the type of *Clostridium botulinum* contaminating the product. For example, proteolytic strains will probably lead to a breakdown of proteins and render the product aesthetically unacceptable (Smith, 1977);
- effective legislation to facilitate identification of potentially toxic foods and preventing them from reaching consumers;
- method of cooking, with temperature and duration affecting the amount of toxin present at time of ingestion;
- dose of toxin ingested and individual susceptibility to the same toxin (Sakaguchi, 1979).

The probability of a fatality resulting from ingestion of the botulinium toxin depends on speed of diagnosis and medical treatment (US Assembly of Life Sciences, 1981).

### 3.2.5 Control of Foodborne Botulism

Food-related botulism can be prevented by any of the following measures:
Chapter 3 \hspace{1cm} \textbf{THE ANTIMICROBIAL FUNCTIONS OF NITRITES & NITRATES}

1) Destruction of spores by adequate heating (applying appropriate temperature-time combination to food being processed).

2) Inhibition of growth of \textit{Cl. botulinium} by:
   - reducing pH (< 4.5), e.g. by adding vinegar;
   - reducing water content, thereby preventing all bacterial growth, e.g. by drying, salting or by adding sugar;
   - refrigerating or freezing to stop bacterial growth;
   - adding ant-botulinal agents (preservatives) such as nitrite.

3.2.6 \textbf{Diagnosis & Treatment}

The onset of symptoms in foodborne botulism is usually 18 to 36 hours (usually within 24 hours) after ingestion of the food containing the toxin, although cases have varied from 4 hours to 8 days (Clayman, Ed, 1994).

Early signs of botulinal intoxication consist of marked lassitude, weakness and vertigo, usually followed by double vision and progressive difficulty in speaking and swallowing. Other common symptoms are breathing difficulty, paralysis of muscles (e.g. eye and throat muscles), constipation, vomiting and intense thirst.

Treatment of the disease is difficult; however early administration of botulinal antitoxin was found to be quite effective, coupled with intensive supportive care including mechanical breathing assistance. Such treatment was in fact found to be instrumental in reducing dramatically the mortality of 1 type of

3.3 Antimicrobial Effect of Nitrite

It is a known fact that nitrite imparts antimicrobial effects against a broad range of bacterial species. However, the mechanisms of this action are not yet fully understood (US Assembly of Life Sciences, 1981).

3.3.1 Effect of Nitrite on Cl. botulinium, & the Perigo Factor

By studying the various factors involved in the production of Shelf-Stable Cured Meats, it was established that nitrite was strongly inhibitory, especially at pH 6.0, the normal pH of canned luncheon meat, and appeared to be the chief preservative against putrefactive anaerobic spoilage.

An important contribution in this field was made by Perigo et al. (1967) who investigated the stability of shelf-stable canned cured meat. It was found that when nitrite was heated in a laboratory medium, an unknown substance was formed that was extremely inhibitory to the growth of vegetative cells of Cl. sporogenes. This inhibitory substance is thought to be derived from nitrite, and disappears (upon reaction) during thermal processing.
Hence the residual nitrite of (shelf-stable) canned cured meat after processing might not reflect the inhibitory capacity of the product. Since the formation of the inhibitor required substantial heating, it was concluded that this inhibitor - called the "Perigo Factor" - played a complementary role in the stability of shelf-stable (but not perishable) canned cured products (Perigo & Roberts, 1968).

The formation of the Perigo Factor in commercially cured meat is highly debatable; and its presence (being highly unstable, low in concentration and functioning in the midst of a complex system e.g. salt, nitrite, pH) is difficult to prove. Yet it appears that nitrite, iron and sulphhydryl groups are involved in the formation of this inhibitor (Pivnick et al., 1969).

Further studies confirmed the interrelation of thermal process, salt, nitrite and spore level in shelf-stable canned cured meat (Pivnick et al, 1969).

In the case of Perishable Cured Meats, stability was assigned to undissociated nitrous acid (rather than the heat-related Perigo factor produced in shelf-stable cured canned meat products). Botulinal growth in such products is controlled by factors such as brine level, pH value, residual nitrite, storage temperature and inoculum level.

With regards to storage temperature, tests carried out on
the effect of vacuum packaging on the safety of such products suggested that perishable cured meat should ideally be frozen to prevent development of microbial health hazards. Data shows that the lowest temperature reported for growth of *Clostridium botulinum* type E on cured meats was 8°C.

Another important factor which increases the significance of nitrite is the growth of competitive flora. In fact, it was found that, for instance, the growth of enterococci in perishable canned hams can inhibit the growth of organisms such as *Clostridium botulinum*, *Clostridium perfringens*, *Clostridium sporogenes* and *Lactobacillus viridescens*.

There are other recognised factors which influence the antibotulinal efficacy of nitrite. These include:

(a) *level of ascorbate or isoascorbate*

addition of ascorbate or isoascorbate can act in concert with residual nitrite to retard botulinal outgrowth in freshly cured products (e.g. bacon) (Tompkin, 1978a). However ascorbate or isoascorbate can also have a negative effect by causing more rapid loss of residual nitrite during processing and storage (Tompkin, 1991). (Hence, the use of ascorbate to inhibit formation of nitrosamines.)
(b) level of “available” iron in product

Nitrite inhibition of *Clostridium botulinum* may be due to a reaction of nitric oxide with an essential iron-containing compound (e.g. ferredoxin) within germinated cells (Tompkin et al., 1979). Such non-haeme iron-sulphur proteins are essential for electron transport, energy production and enzyme activity.

(c) level and type of phosphate

Phosphates do not appear to influence botulinal outgrowths but may play a role through their influence on product pH (Tompkin, 1991).

An important issue addressed by a number of researchers was whether or not the initial concentration of nitrite is more important in providing safety from botulism than the residual nitrite concentrations. Greenberg (1972) and Johnston et al. (1969) found that the initial concentration was more critical than the residual nitrite level. On the other hand, a more recent study indicates that it is the concentration of residual nitrite in cured meat that appears to play a more important role in botulinal control (Christiansen et al., 1978). However, Van Roon & Olsman (1977) emphasize that one must be careful when applying the term “residual” nitrite because low levels of, say, less than 5 mg/kg, can easily be artifacts originating from nitrite released by unstable nitrosated compounds decomposing during the treatment of the sample before analysis.
Other factors which effect the antibotulinal activity of nitrite are:

- temperature of abuse (Wojton et al., 1978);
- level of viable botulinal spores and vegetative cells at the time of abuse (Tompkin, 1978);
- type of meat and other formulation ingredients (Tompkin, 1991);
- the thermal process applied to the product (Tomplin et al., 1978c).

Several other studies were carried out (especially in the 70s) to check also the antibotulinal efficacy of nitrite in vacuum-packaged and fermented meats, and in bacon products.

3.3.2 **Effect of Nitrite on Enteric Pathogens**

Studies on the effect of nitrite on several “enteropathogens” (e.g. *Cl. perfringens*, *E. coli*, *S. typhimurium*, *S. aureus*, *B. Cereus*, etc.) indicated that the nitrite content of cured meats can favour the growth and survival of some of them (e.g. *S. typhimurium*, which can reduce nitrate to nitrite by nitrate reductase, can assimilate inorganic nitrogen from nitrate, nitrite or ammonia, under anaerobic conditions) (Tompkin, 1991).
In fact, it is a known fact that on various occasions, cured meats have been implicated in outbreaks of salmonellosis. This implies that Salmonella can survive, and perhaps proliferate, in a variety of commercially cured products (irrespective of the presence of nitrite) (Tompkin, 1991).

Hence, in order to prevent salmonellosis from cured meat, one must not rely solely on the level of nitrite in the product but on other effective means, especially when one considers that salmonellae can be safely destroyed during processing of most cured meats.

3.3.3 Effect of Nitrite/Nitrate on Yeasts & Moulds

There is no published information, as yet, that nitrite or nitrate inhibit the growth of yeasts and moulds, which were found to occur in commercially cured meats. This is mainly attributed to the number of factors which determine the potential for yeast and mould spoilage of cured meats. Such factors include:

- water activity;
- availability of oxygen;
- the inhibitory effect of smoke;
- storage temperature;
- use of preservatives e.g. sorbate.

Furthermore, yeasts and moulds are of little or no importance to public health since (with a few exceptions) they are not
considered as major causes of spoilage of meat products (Tompkin, 1991).

One specific study (by Obioha et al, 1983) on the effect of nitrite on aflatoxin production by *Aspergillus parasiticus* in fresh pork sausage concluded that high levels of nitrite (e.g. 156-200 µg/g) initially restricted the profuse growth of mould but as the residual nitrite level decreased to less inhibitory levels, the remaining nitrite practically enhanced the growth of the mould and the subsequent production of aflatoxin.

### 3.3.4 Bacteriostatic Mode of Action

The exact mode of action of nitrite as a bacteriostatic agent is still unknown (Kemp, 1974). However, Christiansen et al. (1973) suggested the following 4 possible modes of action.

1. enhancement of heat destruction of spores;
2. increasing the rate of spore germination during thermal processing with subsequent heat-killing of the germinated spores;
3. prevention of growth of the germinated spores which survive thermal processing;
4. reaction with some component of the meat to form an antimicrobial compound.
This may explain why the presence of nitrite in meat, apart from serving as a deterrent to botulism, improves also its shelf-life by preventing non-toxic spoilage from occurring (Kemp, 1974).
Chapter 4

CHEMICAL BEHAVIOUR & ORGANOLEPTIC QUALITIES OF NITRITE IN MEAT PRODUCTS

4.1 Chemical Behaviour of Nitrite

4.1.1 Reactivity of Nitrite during Curing

The curing process is usually carried out by adding mixtures of salt, nitrite, sugar, spices and seasonings together with a reducing agent such as ascorbate or erythorbate to meat. This is usually accompanied by a heating step some time after the initial stages of the curing operation.

Nitrite is a highly reactive species and brings about several reactions when added to the complex meat system. In fact, it is well established that nitrite reacts with myoglobin and several other components in meat including protein and fat (Cassens, 1979).

Nitrites are also known to be water-soluble salts, with the nitrite ion acting as a conjugate base of a weak acid (nitrous acid, HNO₂) having a pKₐ of 3.36. With meat being usually mildly acidic, only a small quantity of nitrous acid is formed (Hotchkiss & Cassens, 1987).
Under different conditions, nitrite may behave both as a weak reducing agent and an oxidising agent. In fact, strong chemical oxidants can oxidise nitrite to nitrate, while nitrite can also oxidise many reduced substances.

Important reactions of nitrite during curing of meat include nitrosation (at the N-, C- or S- atoms), diazotisation and deamination reactions (Ridd, 1961; Challis, 1981). Apart from imparting an important antimicrobial effect (via the presence of nitrite) curing results also in the formation of:

i) a characteristic relatively stable pink colour;

ii) a characteristic flavour (which develops either as a direct result of nitrite or from the effect nitrite has on retarding the development of oxidative-type off-flavours).

Cured meat also acquires a firmer texture than fresh meat; however it is not yet clear whether this is a major function of nitrite or whether it results from the salt used in curing (Hotchkiss & Cassens, 1987).

4.1.2 Antioxidant Properties

Nitrite was found to delay the development of oxidative rancidity in meat. This effect is observed even in the presence of sodium chloride, which tends to promote oxidation of lipids in meat.
It has been suggested by Tarladgis (1961) that nitrite affects rancidity by the same reaction involving the development of the bright pink coloration in cured meat. In fact, when nitrite reacts with haeme compounds to form cured meat pigments, the iron is reduced from $\text{Fe}^{3+}$ (oxidised state, active in lipid oxidation) to $\text{Fe}^{2+}$ (an inactive catalyst).

Herring (1973) noticed that off-flavours increased more rapidly in bacon formulated with low levels ($\approx 15$ ppm) of nitrite, as compared to bacon with higher levels (i.e. $170$ ppm) of the preservative. Similar results were obtained by MacDonald et al (1980a) who found that rancid off-odours and off-flavours in pork were significantly reduced when adding an amount $\geq 50$ ppm of nitrite.

Inhibition of lipid oxidation in cured meats by nitrite is a desirable effect because oxidation of lipids may affect flavour, and the products of such oxidation may pose hazards to human health (US Assembly of Life Sciences, 1982).

4.1.3 Reaction of Nitrite with Myoglobin

An important reaction pathway followed by nitrite is its combination with the muscle pigment myoglobin to form nitrosylmyoglobin. It has been calculated that meat myoglobin binds about 15 ppm of nitrite (Sebranek et al, 1973)
Nitrite may however react also with other porphyrin-containing pigments such as cytochromes and haemoglobin, but such compounds occur in smaller amounts in the muscle compared to the amount of myoglobin. It has also been proposed that under cooking conditions, the pigment may double its binding capacity with nitrite (Tarladgis, 1962). Cassens et al (1974) suggest that about 10-20% of the total nitrite added for curing reacts with porphyrin-containing compounds in the meat.

Figure 3 summarises some of the possible curing reactions of nitrite involving pigment in meat.

Two attributes in cured meats that are usually easily recognised by the consumer are the bright appearance created by the oxymyoglobin in fresh red muscle and the reddish-pink hue of denatured nitrosylmyohaemochrome. Customers tend to make such an association even though the colour of a meat product does not necessarily predict good texture and flavour (Giddings, 1977; Jeremiah et al., 1972).

There are many factors which influence the stability of pigments in meat, one of which being heat. For example, in order to obtain a more stable red pigment in heated commercial meat products, nitrite is added before heating.
Some of the possible curing reactions which occur with the use of nitrite
(Source: Bard & Townsend, 1971)
Chapter 4   CHEMICAL BEHAVIOUR & ORGANOLEPTIC QUALITIES OF NITRITE IN MEAT PRODUCTS

4.1.4 Conversion to Nitrate

Oxidation of nitrite to nitrate in cured meats occurs simultaneously with the oxidation of Fe$^{2+}$ in oxymyoglobin to the Fe$^{3+}$ ion (Mohler, 1967). In fact, bacon formulated without nitrate was found to contain a high content of nitrite with the concentration being even higher when higher levels of nitrite were employed in the curing process (Herring, 1973).

Furthermore, nitrite may also undergo auto-oxidation, resulting in the formation of nitrate and nitric oxide (Smith, 1920).

4.1.5 Conversion to Gases (N$_2$, NO, NO$_2$, N$_2$O)

Nitrous acid may combine with an amino group to produce nitrogen in the so called “van Slyke” reaction. This reaction (which may in fact liberate nitrogen gas (N$_2$) from the meat system, thereby lowering residual nitrite) depends on pH and temperature, and occurs more rapidly under acid conditions and high temperature (with larger proportion of nitrite in the undissociated form) (Cassens, 1974).

Nitric oxide (NO) is the active component for cured colour formation and is one of the key products of the curing process. There are several factors controlling the conversion of nitrite to nitric
oxide and subsequent reactions of the oxide in meat. Such factors probably determine also the residual level of nitrite (Cassens, 1974).

Another gas which may be lost from the meat system is nitrogen dioxide (NO₂) which forms by oxidation of nitric oxide. This reaction depends on the availability of oxygen, which is low in the interior of large cuts of meat (Cassens, 1974).

Nitrous oxide (N₂O) was found to occur in combination with nitric oxide, when nitrite is incubated in the presence of muscle mince (Walters & Taylor, 1964; Walters & Casselden, 1973; Woolford, 1972). Nitrous oxide was also found, together with nitric oxide and nitrogen, in the headspace gas of a heated meat product (Olsman & Krol, 1972).

Although the production and evolution of these gases (i.e. N₂, NO, NO₂, N₂O) by reaction of nitrite with meat may contribute to loss of nitrite in the final product, not all nitric oxide produced in cured meat is necessarily lost as a gas. Some of it remains in the product in combination with a meat constituent or with an added reducing agent (Walters & Casselden, 1973; Cassens, 1974).

4.1.6 Reaction with Sulphydryl (Thiol) Groups

Nitrite was shown to react with sulphydryl groups in meat
to form nitrosothiols (Mirna & Hofmann, 1969), which may, in turn, undergo a redox reaction resulting in a disulphide and the liberation of nitric oxide (which may react further). Considering the fact that the sulphydryl content of meat is about 10 times greater than the amount of nitrite usually added in curing, it could theoretically lead to complete loss of nitrite from the product (Cassens, 1974). In reality only 8-25% of the added nitrite (depending on the heat-treatment given) reacts to form nitrosothiols (Woolford, 1974).

Nitrosothiols formed by reaction of nitrite with sulphydryl compounds, were found to have antibacterial properties. Incze et al (1974) have shown that cysteine-nitrosothiol have a higher microbiological inhibitory effect than nitrite itself.

4.1.7 **Anaerobic Reduction of Nitrite (by Mitochondria)**

Under the right conditions, nitrite was also found to undergo anaerobic reduction by skeletal mitochondria. In fact, it was established that when nitrosylferricytochrome "c" forms during curing, and is incubated with metmyoglobin muscle mitochondria and reduced nictinamide-adenine dinucleotide (NADH) under a nitrogen atmosphere, the products would be equal amounts of ferrocytochrome "c" and nitrosylmyoglobin (with nitrosylmet-myoglobin as the intermediate product) (Walters et al, 1967; Cassens, 1974).
4.1.8 **Effect on Meat Texture**

The texture of meat is probably altered by reaction of nitrite with non-haeme proteins. However, this is difficult to prove due to extreme sensitivity in measurements of the reactions with the protein and due to the fact that salt also plays a major role in determining the physical properties of the cured product.

Studies on texture of meat carried out by Randall & Voisey (1977) concluded that the texture of some products such as ham and frankfurters was practically unaffected by the addition of nitrite.

4.1.9 **Other Reactions**

Loss of nitrite in cured meats could also be partly attributed to a number of other possible reactions:

1. **With Ascorbate**

Nitrite is known to react with reducing agents such as ascorbate. Such interaction is important since ascorbate is usually added in the curing process and may even occur endogenously in small amounts in meat. It was also found that decomposition of nitrite, in the presence of ascorbate, is enhanced by certain metal ions (such as Mg$^{2+}$, Ca$^{2+}$, Zn$^{2+}$, Fe$^{2+}$, Fe$^{3+}$) (Ando, 1974).
(2) **Nitroso Intermediates/Products**

Other nitrite losses may be due to the formation of nitroso compounds including stable intermediates. A number of volatile nitrosamines were found in cured food, an example being nitrosopyrrolidinedine in cooked bacon. Possible pathways for nitrosamine formation are still under discussion (US Assembly of Life Sciences, 1981).

Non-volatile nitrosamines may also form and may even occur in meat in higher amounts than the volatiles. However the extent of their effect on residual nitrate is still being investigated.

(3) **With Lipids**

Nitrite may even react with lipid components of meat but little is known on such reaction. However it seems that such reaction retards lipid oxidation or the development of warmed-over flavour (WOF) in cooked meat and meat products. Frouin et al (1975) proved that nitric acid reacts with unsaturated fatty acids present in adipose tissue in meat.

(4) **Black Roussin Salt & Perigo Factor**

Iron, nitric oxide (NO) and sulphur may form a complex coordination compound, known as black Roussin salt (a possible
microbial inhibitor) leading to further loss of nitrite (Van Roon, 1974; Mirna, 1974; Walters, 1974). Another possible route for nitrite disappearance is the antibacterial Perigo factor discussed in section 3.3.1 (Perigo et al., 1967).

(5) **Radiation Sterilisation**

It has been reported (Wierbicki et al., 1974) that meat products preserved by irradiation (radappertisation) required lower amounts of curing agents to maintain characteristic colour, flavour and control of *Cl. botulinium*. The effect of radiation on nitrite is however not yet fully understood.

4.1.10 **Overall Fate of Nitrite**

Following several studies on the reactions of nitrite in meat products, Cassens et al. (1977) arrived at generalising and quantifying the fate of nitrite as follows (Table 8), with values representing percentage of the nitrite originally added:

<table>
<thead>
<tr>
<th>Added Nitrite</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-20 %</td>
<td>nitrite</td>
</tr>
<tr>
<td>1-10 %</td>
<td>nitrate</td>
</tr>
<tr>
<td>1-5 %</td>
<td>gases</td>
</tr>
<tr>
<td>5-15 %</td>
<td>sulphhydryl compounds</td>
</tr>
<tr>
<td>1-5 %</td>
<td>lipid</td>
</tr>
<tr>
<td>20-30 %</td>
<td>protein</td>
</tr>
<tr>
<td>10-20 %</td>
<td>myoglobin</td>
</tr>
</tbody>
</table>
It could be noted that heated myoglobin binds approximately twice as much nitrite as non-heated myoglobin. This suggests that both the free coordination positions of Fe are occupied by nitric oxide in the presence of nitrite in heated samples (Lee & Cassens, 1976).

Frouin (1977) suggested that the crucial reaction determining the fate of nitrite in meat is its rapid breakdown into nitric oxide (NO) since this oxide can be fixed and kept in solution by many compounds occurring in meat. Figure 3 shows the whole reaction scheme proposed by Frouin.

![Figure 4](image_url)

**Figure 4**

Probable scheme of reaction of nitrite in meat products
(Source: Frouin, 1977)
The same source claims that all nitrite is probably reduced to NO. Being highly reactive, this oxide becomes fixed to some degree to many substances found in meat such as proteins, thiols, hydroxyls, carboxyls, reducing agents and haematic compounds. Such substances (that can fix NO with variable force) form a complex equilibrium.

It has also been proposed that this equilibrium is upset during analysis by the reagents, which release some of the NO (depending on the conditions). Reducing agents, e.g. cadmium, cause more NO to be freed (Frouin, 1977). Other interactions may also take place during extraction and filtration (Mirna & Hofman, 1969).

All this explains why the analysis of nitrite is difficult and why nitrosamines form only in certain conditions.

4.2 Organoleptic Aspects of Nitrite

Apart from acting as an antimicrobial agent and antioxidant, sodium nitrite is also responsible for the development of the typical cured meat colour and flavour.
4.2.1 Colour Development

Cured meat colour develops as a result of reaction of sodium nitrite with haeme pigments in the muscle. It is considered as the most obvious effect of adding nitrite to meat. Such reaction involves 2 main steps.

- **Step 1** involves reduction of nitrite to nitric oxide (very unstable) accompanied by reduction of the iron in myoglobin (haeme pigment) to the Fe$^{2+}$ (ferrous) state.

- **Step 2** involves denaturing of the protein part of the myoglobin by heat during thermal processing to form a much more stable compound - nitrosohaemochrome.

In the USA, a dilemma cropped up on the classification of nitrite as a food additive. The problem was whether nitrites served as *colour “additives”* (by ‘impartment’ colour to bacon and other red meats) or *colour “fixatives”* or “stabilizer” (as suggested by the American Meat Institute Foundation, 1973).

In general, only a small fraction of the nitrite added to a meat product is utilised for colour fixation. In theory, it is calculated that only 3 mg/kg sodium nitrite are required to provide a 50% conversion of myoglobin to nitrosylmyoglobin (MacDougall et al,
1975). However, more is required to provide colour stability (due to effects of many factors that might be involved in the meat system) and to make up for losses due to reaction of nitrite with other meat components (e.g. amino groups, sulphydryl groups).

4.2.2 Flavour Development

Although addition of nitrite brings about a definite modification of the fresh meat flavour, little is known to date about the mechanism of the reactions leading to the formation of the “cured meat flavour” or of the types of volatile and non-volatile compounds responsible for such flavour.

4.2.2.1 Precursors of Meat Flavour

The overall flavour sensation of meat derives from a combination of volatile compounds during the cooking of meat. Herz and Chang (1970) suggest that heat is also instrumental:

(i) to release flavour precursors from fat structures;

(ii) to allow intimate mixing of fat-soluble and water-soluble components;

(iii) to accelerate browning reactions.

Meat flavour derives from non-volatile components which are water-soluble as well as fat-soluble. Such precursors are probably low molecular weight compounds such as glycoproteins, reducing
sugars, amino acids and their degradation products. It is also known that meat flavour per se (which resides in the water-soluble fraction of meat) is similar in all species, with species differences arising from the lipid fraction (which, therefore, contains flavour that discriminates between pork and beef or fish) (Hornstein, 1967; Hornstein et al., 1960).

Lipids in fact, affect the overall flavour of all foods. They modify the odour and flavour of other components, many having odours and flavours themselves and serving as precursors for other flavours (Chang & Peterson, 1977).

In spite of the large number of components found in meat (via GC and MS techniques), no single compound or group of compounds has yet been attributed the characteristic flavour of meat. The same applies to the characteristic "cured" aroma of cured meat (Chang & Peterson, 1977; Herz & Chang, 1970).

4.2.2.2 "Cured" Aroma

Recent attempts to identify the volatile components produced during cooking of cured meats have been unsuccessful. However, sensory studies with such products revealed some kind of relationship of cured meat flavour to the concentration of nitrite added to meat.
In fact, Mottram & Rhodes (1974) found that added nitrite levels of less than 200 ppm were adequate to produce the typical cured meat flavour in bacon. On the other hand, MacDonald et al. (1980b) claimed that concentrations of nitrite as low as 50 ppm produced significant cured meat flavour in ham.

There are other factors (apart from nitrite) that can contribute to cured meat flavour. These include:

- addition of sodium chloride;
- smoking;
- addition of spices and other flavouring agents;
- processing.

Given the presence of so many variables in the type of meat product and processing, the contribution of nitrite to the cured meat flavour must be better assessed on a product-by-product basis.
5.1 **Occurrence of Nitrite & Nitrate in Food**

Nitrates are continuously consumed in substantial amounts with food. In fact, they are found to occur in vegetables, meat and other food at concentrations of up to several grams per kilogram.

Consumption of nitrites and nitrates is likely to increase due to their presence as antimicrobial agents in meat products at levels ranging from tens (in the case of nitrite) to hundreds (in the case of nitrate) of milligrams per kilogram of product. In the case of certain types of cheese, nitrates may also reach the level of several tens of milligrams per kilo.

5.2 **The Origins of Nitrite and Nitrate**

Plants usually take up inorganic nitrogen in the form of nitrate or ammonia, to synthesize proteins and other nitrogenous
organic matter. This is carried out either by direct absorption of the ions from the soil or (in the case of legumes) by bacterial fixation of atmospheric nitrogen in root nodules to produce nitrate. If excess nitrate is available in the plant, it may be taken up and retained, unless it is metabolised by any synthetic process. Hence the nitrate content of vegetable products is a result of:

a) cultivation (agricultural practice);
b) fixation of atmospheric nitrogen (natural process).

On the other hand, the presence of nitrate (and nitrite) in food products of animal origin (such as meat and cheese) is mainly attributed to human addition or, in the case of fermented meat products, production process.

Organic nitrogen, present in vast quantities in the soil, is slowly converted to inorganic salts by a series of biological processes (nitrogen cycle) the final stages being the formation of the $\text{NH}_4^+$, $\text{NO}_2^-$ and $\text{NO}_3^-$ ions. Oxidation of ammonium to nitrite is very fast, except at very low temperatures, while oxidation of nitrite to nitrate occurs so rapidly that nitrite remains practically undetectable in soils.

Although nitrate may be reduced in the root of certain plants, reduction usually takes place in the leaves. In fact, crops with a rich supply of nitrogen often accumulate high concentrations of
nitrate in their leaves. Control on nitrate content in plants is partly achieved by an enzyme, nitrate reductase, which is present in the soil and requires a molybdenum co-factor.

The soil is usually replenished with nitrogen via organic matter such as crop residues and manures, and inorganic matter such as ammonium, nitrate and urea fertilisers. Other sources of nitrogen include rainwater and bacterial fixation of atmospheric nitrogen. The increased use of nitrogen-containing fertilisers in recent years has probably been the main factor resulting in:

(i) an increase in nitrate concentration in some crops (especially leaf crops);
(ii) an increased loss of nitrogen from the soil.

Nitrogen can be lost from the soil in one of two ways:

* by volatilisation
  (as ammonia, nitrous oxide N₂O and nitrogen), and
* by leaching.

The ammonium ion is usually bound by anions present in soil particles and so has low mobility; hence, it resists leaching. On the other hand, nitrite remains in soil solution and so can be leached below the depth to which roots penetrate, ultimately passing into ground water.
Nitrate may also be lost via run-off of surface water. Loss of nitrate through leaching and surface water run-off is highest during winter. Grazing seems to affect loss of nitrate by leaching in grassland with low losses being registered in low intensity grazing with respect to greater losses in intensively grazed systems.

5.3 Nitrite & Nitrate in Food & Drinking Water

In order to control intake of nitrite and nitrate by humans, the sources must be identified and understood. According to the report of dietary nitrite intake by the US Assembly of Life Sciences (1981), nitrite originates from:

- cured meat, 39%;
- baked goods and cereals, 34%
- vegetables, 16%

On the other hand, the main sources of nitrate in the diet are vegetables, contributing about 75-80% of the total daily intake. Other important sources are meat and meat products, milk and dairy products, fish and fish products, cereals and cereal products, fruits and fruit juices, and water.

5.3.1 Vegetables

The highest concentrations of nitrate in the diet and of
dietary intakes were found in vegetables, with concentrations varying widely from one type to another (from tens to thousands mg/kg fresh weight). In contrast, nitrite concentrations in fresh vegetables rarely exceed 2 mg/kg although this amount may be exceeded on improper storage. Table 9, based on figures published by White (1975, 1976) and Corre & Breimer (1979), summaries average concentrations of nitrate and nitrite in vegetables.

5.3.2 **Meat and Meat Products**

Fresh meats usually contain only low concentrations of nitrate and nitrite. However, nitrate and in particular nitrite are added as antimicrobial ingredients in preparations of cured meat. Nitrate is believed to serve as a “nitrite reservoir” since it can be partially converted to nitrite by microbiological and biochemical processes occurring in meat.

Surveys of the nitrate and nitrite concentrations of cured meats have revealed a radical decrease in maximum observed levels of nitrate from 6,000 mg/kg in 1959 to 1,000 mg/kg in 1972 and 450 mg/kg in 1975 (US Assembly of Life Sciences, 1981; UK Ministry of Agriculture, Fisheries & Food, 1978). EC Legislation has brought down maximum levels of nitrate to 250 mg/kg in the 1990s. A parallel reduction in nitrite, from levels frequently above 100 mg/kg before 1970 to below 50 mg/kg in 1973/74, was also observed (Walker, 1990).
## Table 9

### Average Concentrations of Nitrate and Nitrite in Vegetables

*(Source: Walker R., 1990)*

<table>
<thead>
<tr>
<th>Vegetable</th>
<th>Nitrate (mg/kg fresh weight)</th>
<th>Nitrite (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artichoke</td>
<td>16</td>
<td>0.6</td>
</tr>
<tr>
<td>Asparagus</td>
<td>60</td>
<td>0.9</td>
</tr>
<tr>
<td>Aubergine</td>
<td>370</td>
<td>0.8</td>
</tr>
<tr>
<td>Beans: Green</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lima</td>
<td>74</td>
<td>1.7</td>
</tr>
<tr>
<td>dry (navy)</td>
<td>18</td>
<td>--</td>
</tr>
<tr>
<td>Beet</td>
<td>3288</td>
<td>6.0</td>
</tr>
<tr>
<td>Broccoli</td>
<td>1014</td>
<td>1.5</td>
</tr>
<tr>
<td>Brussels sprouts</td>
<td>164</td>
<td>1.5</td>
</tr>
<tr>
<td>Cabbage</td>
<td>712</td>
<td>0.8</td>
</tr>
<tr>
<td>Carrot</td>
<td>274</td>
<td>1.2</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>658</td>
<td>1.7</td>
</tr>
<tr>
<td>Celery</td>
<td>3151</td>
<td>0.8</td>
</tr>
<tr>
<td>Corn</td>
<td>62</td>
<td>3.0</td>
</tr>
<tr>
<td>Cucumber</td>
<td>151</td>
<td>0.8</td>
</tr>
<tr>
<td>Endive</td>
<td>1780</td>
<td>0.8</td>
</tr>
<tr>
<td>Kale</td>
<td>1096</td>
<td>1.5</td>
</tr>
<tr>
<td>Leek</td>
<td>700</td>
<td>--</td>
</tr>
<tr>
<td>Lettuce</td>
<td>2330</td>
<td>0.6</td>
</tr>
<tr>
<td>Melon</td>
<td>4932</td>
<td>--</td>
</tr>
<tr>
<td>Mushroom</td>
<td>219</td>
<td>0.8</td>
</tr>
<tr>
<td>Okra</td>
<td>52</td>
<td>1.1</td>
</tr>
<tr>
<td>Onion</td>
<td>235</td>
<td>1.1</td>
</tr>
<tr>
<td>Parsley</td>
<td>1380</td>
<td>--</td>
</tr>
<tr>
<td>Peas</td>
<td>40</td>
<td>0.9</td>
</tr>
<tr>
<td>Pepper: sweet</td>
<td>165</td>
<td>0.6</td>
</tr>
<tr>
<td>Potato: white</td>
<td>150</td>
<td>0.9</td>
</tr>
<tr>
<td>sweet</td>
<td>65</td>
<td>1.1</td>
</tr>
<tr>
<td>Pumpkin, squash</td>
<td>550</td>
<td>0.8</td>
</tr>
<tr>
<td>Radish</td>
<td>2600</td>
<td>0.3</td>
</tr>
<tr>
<td>Rhubarb</td>
<td>2900</td>
<td>--</td>
</tr>
<tr>
<td>Spinach</td>
<td>2470</td>
<td>3.8</td>
</tr>
<tr>
<td>Tomato</td>
<td>80</td>
<td>--</td>
</tr>
<tr>
<td>Turnip: root</td>
<td>535</td>
<td>--</td>
</tr>
<tr>
<td>greens</td>
<td>9040</td>
<td>3.5</td>
</tr>
</tbody>
</table>
The following table (Table 10) shows the mean values for nitrate and nitrite concentrations found in a number of surveys on cured meat products.

**Table 10**

Range of mean nitrate and nitrite contents of cured meat products  
(Source: Walker R., 1990)

<table>
<thead>
<tr>
<th>Meat Product</th>
<th>Nitrate (as NaN0₃)</th>
<th>Nitrite (as NaN0₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preserved meat</td>
<td>19-49</td>
<td>5-39</td>
</tr>
<tr>
<td>Corned Beef (canned)</td>
<td>60-70</td>
<td>15.23</td>
</tr>
<tr>
<td>Pork luncheon meat</td>
<td>107-205</td>
<td>9-23</td>
</tr>
<tr>
<td>Raw ham</td>
<td>204-470</td>
<td>21-31</td>
</tr>
<tr>
<td>Cooked ham</td>
<td>1295</td>
<td>22</td>
</tr>
<tr>
<td>Canned ham</td>
<td>275</td>
<td>22</td>
</tr>
<tr>
<td>Chopped ham &amp; pork</td>
<td>110</td>
<td>15</td>
</tr>
<tr>
<td>Bacon</td>
<td>77-235</td>
<td>40-90</td>
</tr>
<tr>
<td>Sausage/sausage meat</td>
<td>19-670</td>
<td>0-96</td>
</tr>
<tr>
<td>Tongue (cured)</td>
<td>70</td>
<td>15</td>
</tr>
</tbody>
</table>
5.3.3 Milk & Dairy Products

Whole milk usually contains a low concentration of nitrate which rarely exceeds 5 mg/L. According to the US Assembly of Life Sciences (1981), this is not much affected by the animals' diet.

Naturally-derived nitrate in cheeses usually ranges from 1-8 mg/kg. However cheeses made with specific addition of nitrate may contain somewhat higher levels, e.g. 30-66 mg/kg (as sodium nitrate) in certain Dutch cheese and 4-27 mg/kg nitrate in English Edam cheese.

5.3.4 Fish & Fish Products

Not much data is available on the levels of nitrate/nitrite in fish and fish products, although they are permitted additives in some countries.

Italian surveys (Cantoni & Maccapani, 1974, and Cantoni et al., 1978) found nitrate concentrations in fresh fish to be mainly in the range of 5-30 mg/kg with occasional fish containing up to 65 mg/kg; nitrite level was found in the range of 1-5 mg/kg but occasionally higher with a maximum figure of 13 mg/kg. Higher levels of nitrate (maximum 380 mg/kg as sodium nitrate) were found in certain fish food during a total diet study carried out in the UK in 1979.
5.3.5 Cereal & Cereal Products

Cereal grains usually contain low nitrate and nitrite content, depending on species, strain and growing conditions. A typical example is winter wheat with nitrate concentration reported to be in the region of 0.5-15 mg/kg (McNamara et al., 1971). The concentration of nitrate and nitrite in wheat flour was found to increase from 1-2 mg/kg to an average of 18 mg/kg (for nitrate) and 5 mg/kg (for nitrite) after baking (Selenka & Brand-Grimm, 1976).

The mean nitrate concentration in the cereal food group is reported as 12 mg/kg in the UK (UK Ministry of Agric., Fisheries & Food, 1987) and 16 mg/kg in the USA (US Assembly of Life Sciences, 1981) with a mean nitrite level of 4 mg/kg in the latter case.

5.3.6 Fruits & Fruit Juices

Fruits contain less than 10 mg/kg of nitrate. Exceptions were found to be bananas, strawberries and tomatoes with levels occasionally as high as 150 mg/kg (Achtzehn & Hawat, 1969). Nitrite content in fruits is usually negligible (< 1 ppm) (Harada et al., 1972; UK Ministry of Agric., Fisheries & Food, 1987).

5.3.7 Water

Drinking water supplies are usually derived from both surface and ground water, the proportion of each varying from
country to country. About 2% of European population draws water from shallow private wells (ECETOC, 1988)

The concentration of nitrate in drinking water varies widely depending on the source, season and proximity to arable land. In Norway, for example, about 85% of the population receive surface water with very low concentration of nitrate (about 2 mg/L); ground water supplies rarely exceed 9 mg/L. On the other hand, maximum nitrate concentrations higher than 100 mg/L were recorded in a few supplies of drinking water in countries such as Bulgaria, Italy, Netherlands and France (Walker, 1990).

Nitrite is only found in trace amounts in drinking water, except in grossly polluted well waters (Walker, 1990).

5.4 Estimated Dietary Intakes of Nitrite & Nitrate

There are 3 techniques through which estimates of daily intakes of nitrate and nitrite have been made (Gangolli, et al., 1994). These are:

(i) duplicate diet analysis (as published by WHO, 1985) which is very difficult to perform to obtain representative data, but which takes into account seasonal variations and changes occurring
during preparation and cooking;

(ii) analysis of all components of the diet and calculating the intake from food consumption data; and

(iii) calculating the intake from published mean values for nitrate content of dietary components and data consumption.

Such techniques were all used to arrive at the estimates of daily intakes of nitrate and nitrite shown in table 11.

Table 11

Estimated Dietary Intake of Nitrate and Nitrite by Analysis & Calculation
(Source: Walker R., 1990)

<table>
<thead>
<tr>
<th>Country</th>
<th>Mean daily intake (mg/day)</th>
<th>Nitrate</th>
<th>Nitrite</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>150-7a</td>
<td>&gt; 3</td>
<td></td>
</tr>
<tr>
<td>Federal Rep. of Germany</td>
<td>68a</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>245</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Netherlands</td>
<td>71</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Norway</td>
<td>43</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>Poland: adult</td>
<td>178</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>child 1-3yr</td>
<td>142</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>infant 3-12mth</td>
<td>30</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Switzerland</td>
<td>125</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>U.K.</td>
<td>102</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>U.K.</td>
<td>88-407</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>U.K.</td>
<td>97</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>U.K.</td>
<td>84</td>
<td>0.3-0.9</td>
<td></td>
</tr>
<tr>
<td>U.K.</td>
<td>149</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>U.S.A: normal diet</td>
<td>103</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>high cured meat</td>
<td>107</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>vegetarian</td>
<td>367</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>nitrate-rich water</td>
<td>319</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>U.S.A</td>
<td>137</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>U.S.A.</td>
<td>53</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>U.S.A.</td>
<td>96</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>U.S.A.</td>
<td>137</td>
<td>0.9</td>
<td></td>
</tr>
</tbody>
</table>
As previously said the main sources of dietary nitrate are vegetables, with more than 75% of the total dietary intake usually coming from this source (White 1975, UK Ministry of Agriculture, Fisheries & Food, 1987) and, with total intake of nitrate being higher in vegetarian or vegan diets (US Assembly of Life Sciences, 1981).

In regions with relatively high nitrate concentrations in drinking water, a significant proportion of the daily intake may derive from such source. In the case of neonatal infants, breast-fed babies have a low nitrate intake while bottle-fed babies receive nitrate primarily from drinking water.

Dietary intakes of nitrite are low, usually less than 2 mg NaNO₂/day, the major source being cured meats. Smaller amounts may be found in fish and cheese products. Other possible sources are cereal products and vegetables. Nitrite may form during storage and/or cooking of such products (Walker, 1990).

In addition of the diet, nitrates and nitrites may also arise from nitrogen oxides (nitrous oxide N₂O, nitric oxide NO and nitrogen dioxide NO₂) present in the atmosphere and especially in tobacco smoke, which may add a further 4 mg nitrate/day to the intake of smokers (Walker, 1990). Nitrate may also be biosynthesized in mammals via metabolic pathways.
5.5 Acceptable Daily Intake (ADI)

The ADI is defined as the amount of a chemical, expressed on a mg/kg body weight basis, which can be ingested daily over a lifetime, without incurring any appreciable health risk. It is usually based on an evaluation of available toxicological data.

Taking into account the various possible sources, health risks and benefits or nitrate and nitrite in the human diet, data from experimental animals and observations in man, the Scientific Committee for Food (SCF) on Nitrate and Nitrite of the Commission of European Communities (EC), in its 1992 report, established the following limits of daily intakes (Commission of European Communities, 1992):

(i) an Acceptable Daily Intake (ADI)
of 0 to 5 mg of nitrate per kg body weight (expressed as sodium nitrate);

(ii) a “Temporary” Acceptable Daily Intake
of 0 to 0.1 mg of nitrite per kg body weight (expressed as sodium nitrite). This ADI is considered temporary pending clarification of the mechanism of adrenal effects of potassium nitrite observed in a recent short-term rat study (Commission of European Communities, 1992).
Such values are very close indeed to the levels set forth by the FAO/WHO (World Health Organisation, 1978) when quoting an ADI of 220 mg nitrate and 8 mg nitrite for a typical 60 kg person (Schuddeboom, 1993).

ADI values were lately adjusted by the SCF in 1995, establishing maximum levels (rather than ranges of values) as follows (EC Scientific Committee for Food, 1995):

i) 3.7 mg/kg body weight for nitrate;

ii) 0.06 mg/kg body weight for nitrite.

It should be noted that the ADIs for nitrate and nitrite incorporate large safety factors which serve as protective measures against the hazard arising from intakes that are close to or inadvertently exceed ADIs (UK Ministry of Agric., Fisheries & Food, 1997, 1998). Figures include human intake from all possible sources.

The SCF (EU Scientific Commission for Food, 1995) further specifies that ADI values are not to apply to infants (under 3 months of age) since these are:

a) more prone to reduce oxogenous nitrate to nitrite;

b) more sensitive to the acute effects of nitrite.

In fact, nitrate is not permitted as an additive in infant foods.
5.6 Endogenous Synthesis & Distribution of NO$_2^-$ & NO$_3^-$ in Meat

Apart from being consumed with food, nitrate is also produced endogenously through the metabolism of the nitrogen pool in the body tissues and in the gut microflora as shown in the Figure 5.

It has been estimated that the rate of endogenous synthesis of nitrite in the newborn and in the adult, as well as in experimental animals, is about 1 mg/kg body weight/day (Green et al., 1981, Bartholomew & Hill, 1984). Synthesis of nitrite is normally associated with tissue metabolism; however, rate of biosynthesis was found to increase by five times in adults and even by 15 times in newborns in cases of acute enteritis (Hegesh & Shiloah, 1982; Wettig et al., 1987).

Once ingested, nitrate is absorbed readily through the small intestine and is distributed rapidly via blood circulation, to the tissues, including the kidneys, the latter being the major site of excretion of this ion. An interesting aspect of nitrate distribution is its secretion by an active transport system located in the salivary glands. In fact, it was determined that in man, about 25% of the ingested nitrate recirculates into the oral cavity by means of salivary secretion (Spiegelhalder et al., 1976, Tannenbaum et al., 1976).
Figure 5

Circulation of endogenous & exogenous nitrate and nitrite in mammals
(Source: Vittozzi, 1992)

N.B. Rats lack the active transport system for nitrate secretion in saliva.
In rats, the gastric pH is 4-5, while in humans, it is 1-2. Excretion of nitrate through milk in humans is lower than in rats.
Salivary secretion of nitrate in man allows its conversion to nitrite by the microflora present in the oral cavity and in the hypochlorhydric stomach. Reduction of salivary nitrate to nitrite has been quantified and was found to be in the region of 20%, i.e. about 5% of ingested nitrate (Eisenbrand et al., 1980; Stephany & Schuller, 1980).

Such findings cannot be compared with those of rats in which production of nitrite takes place mainly in lower intestinal tract, where secretion occurs and an abundant microflora is present. There are also substantial differences in pH values of gastric juice of rats and humans (Sen et al., 1969). Hence, toxicological studies based on exposure of rats to nitrate do not allow a reliable evaluation of the effects of the oro-gastric formation of nitrite in man.

5.7 Toxic Effects of Nitrate & Nitrite

5.7.1 Methaemoglobinemia

Toxicity of nitrate in humans, as well as in animals, depends on the conversion of nitrate to nitrite (JECFA, 1996b). Ingestion of a single dose or continuous ingestion (up to 2 years) or either substance results in methaemoglobinemia (Vittozzi, 1992). The condition is characterized by cyanosis and anoxia, and results
from defective transport of oxygen by high levels of circulating methaemoglobin (Clayman, 1994).

Methaemoglobin is the product of a redox reaction between haemoglobin and nitrite, in which the Fe$^{2+}$ ion of haemoglobin is oxidised to the Fe$^{3+}$ form. Such reaction brings about a reduced oxygen supply to body tissues because oxygen can no longer reversibly bind to red blood cells. If oxidation damage proceeds far enough, the haemoglobin may be irreversibly damaged, leading to the formation of haemochromes. Haemoglobin may finally be denatured and precipitated (as Heinz bodies) leading to the death of the red blood cells.

Acute cases of intoxications have been reported due to drinking of well water containing high nitrate levels (World Health Organisation, 1978).

Infants are at a greater risk of developing methaemoglobinaemia from excessive intake of nitrate and nitrite (Walker, 1990; Lee, 1970). Such increased susceptibility is attributed to at least 4 factors:

1) Foetal haemoglobin is much more readily oxidised than haemoglobin in adults.
2) Newborns have a deficiency of methaemoglobin reductase (or its
cofactor, NADH - reduced nicotinamide adenine dinucleotide) which can reduce methaemoglobin back to functionally active haemoglobin.

(3) On a weight basis, infants consume approximately 10 times more water than adults.

(4) Reduction of nitrate may be enhanced by gastric or intestinal microflora because of the low gastric acid secretion or because of acute gastroenteritis (Lee, 1970).

5.7.2 Miscellaneous Effects

Excessive intake of nitrate esters (vasodilators, used to treat angina pectoris) may induce headache, facial flushing and, in severe cases, even fainting and hypotension. Chronic exposure to high levels of such esters may lead to the development of tolerance to the vascular effect (e.g. workers in nitroglycerin factories).

It is reported that an oral therapeutic does of sodium nitrite (0.03-0.12 g) may be used for vasodilation; however, an amount of 1g of this salt is considered as a lethal dose (Cassens, 1995).

A potentially serious effect of chronic exposure to nitrate esters is the development of dependence on organic nitrate derivatives (e.g. explosive workers found to recover rapidly from myocardial ischaemia/infarction after treatment with nitroglycerine).
5.8 The Chemistry of Nitrosation & its Toxicological Consequences

Nitrite may form nitroso compounds by reaction with nitrosatable compounds which may be food components (e.g. amines, free amino acids and proteins) and other substances including pesticide residues or drugs (Vittozzi, 1992). In the case of amines, amides or ureas, this may result in the formation of N-nitrosamines, N-nitrosamides and N-nitrosoureas respectively, having the following general structures.

\[
\text{N-nitrosamine} \quad \text{N-nitrosamide} \quad \text{N-nitrosourea}
\]

\[
\begin{align*}
\text{Figure 6} \\
\text{Generalised structures of N-nitroso compounds} \\
\text{(Source: Vittozzi, 1992)}
\end{align*}
\]

where \( R_1, R_2 = \text{alkyl, aryl or part of a cyclic structure} \)
The chemistry of nitroso compounds has been reviewed in detail by Fridman et al. (1971) and Challis (1981). Nitrosamines tend to be more stable than nitrosamides. In fact:

- N-nitrosamines were found to be relatively stable under most conditions found in foods (Fan & Tannenbaum, 1972) and do not decompose during food processing or preparation;

- Nitrosamides are considered to be much less stable, especially at neutral to basic pH. Kakuda et al. (1980) have determined that it is unlikely for nitrosamides to survive common cooking procedures.

The nitrosation reaction involves several steps. Nitrite cannot react directly with amines, but must first be converted to nitrous acid (HNO₂) and/or nitrous anhydride (N₂O₃). Figure 7 shows that this conversion is favoured by acidic conditions. However, if the conditions are too acidic, the amine will be protonated and not able to react with the nitrous anhydride (N₂O₃). This implies that nitrosation occurs most rapidly at some optimum pH, usually pH 2-4 for most common amines in food.
Figure 7

Mechanism of nitrosation of secondary amines and amides
(Source: Council of Europe, 1995)
Hotchkiss & Cassens (1987) argue that there might be other important conditions affecting nitrosation. For example certain oxides of nitrogen ($\text{NO}_x$) can react directly with amines without the requirement for acid as in the case of nitrite. Furthermore, some components found in foods may catalyse nitrosation reactions. The same authors believe that both acidic and non-acidic nitrosation mechanisms play an important role in the occurrence of nitrosamines in foods.

Such N-nitroso compounds (which may be volatile or non-volatile) may be formed in certain foods under conditions where the nitrosating agent (nitrite or nitrogen oxides) can react with amines or amides naturally present in food. Nitrosating agents can also be formed in vivo, under certain conditions:

**Volatile N-nitrosamines**

The most commonly occurring volatile N-nitroso-compounds in foods are:

- N-nitroso dimethyl amine (NDMA);
- N-nitroso diethyl amine (NDEA);
- N-nitroso pyrrolidine (NPYR); and
- N-nitroso piperidine (NPIP).
Table 12 shows the concentrations of such compounds in various foods. From this table one may note that the most significant sources of N-nitroso compounds are cured meats (especially fried bacon), malt-based fermented beverages and in some countries, fish. Such levels have however been reduced considerably since 1970s with changes in manufacturing technology.

### Table 12

**Major Sources of Dietary Nitrosamines.**

(Source: Walker R., 1990)

<table>
<thead>
<tr>
<th>Food</th>
<th>Concentration of nitrosamines (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NDMA</td>
</tr>
<tr>
<td>Bacon (fried)</td>
<td>n.d.-30</td>
</tr>
<tr>
<td>Smoked meats</td>
<td>n.d.-3</td>
</tr>
<tr>
<td>Sausages:</td>
<td></td>
</tr>
<tr>
<td>Frankfurter</td>
<td>n.d.-84</td>
</tr>
<tr>
<td>Mettwurst</td>
<td>+</td>
</tr>
<tr>
<td>Salami</td>
<td>n.d.-80</td>
</tr>
<tr>
<td>Bologna</td>
<td>---</td>
</tr>
<tr>
<td>Fish:</td>
<td></td>
</tr>
<tr>
<td>* Oriental salt-fried</td>
<td>40-9000</td>
</tr>
<tr>
<td>* Smoked nitrate or nitrite cured</td>
<td>4-26</td>
</tr>
<tr>
<td>* Fresh, smoked or salted, UK</td>
<td>1-9</td>
</tr>
<tr>
<td>* Crude salted, Hong Kong</td>
<td>up to 400</td>
</tr>
<tr>
<td>* Cooked fish and fish products</td>
<td>n.d.-45</td>
</tr>
<tr>
<td>Fishmeal</td>
<td>120-100,000</td>
</tr>
<tr>
<td>Dairy products:</td>
<td></td>
</tr>
<tr>
<td>Cheeses (various)</td>
<td>n.d.-20</td>
</tr>
<tr>
<td>Dried skimmed milk</td>
<td>n.d.-4.5</td>
</tr>
<tr>
<td>Beer</td>
<td>n.d.-68</td>
</tr>
</tbody>
</table>

*n.d.* = not detected;  * + = detected but not quantified
♦ **Non-Volatile N-nitrosamines**

Cured meats may also contain non-volatile N-nitrosamines. The presence of these compounds in food has not been widely reported since their non-volatile character does not facilitate their isolation from foodstuffs. One of the few non-volatile N-nitroso compounds which have been recently reported to occur in model and cured meat systems is 3-hydroxy-1-nitrosopyrrolidine (HN-Pyr).

Figure 8 (Schuddeboom, 1993) compares the possible routes of human exposure to nitrates, nitrites and N-nitrosamines.

**Figure 8**

Possible routes of human exposure to nitrates, nitrites & nitrosamines

(Source: Schuddeboom, 1993)
Nitrosation can occur in 2 ways (Vittozzi, 1992):

(i) during storage and ripening of food product;
(ii) in the stomach due to the action of salivary nitrite produced via enzymatic reduction of endogenous or exogenous nitrate.

Over the years, there was an increasing public concern over the presence of nitrosamines in certain cured meat products due to their potential carcinogenicity (Gray & Randall, 1979). Studies on test animals show that nitrosation is related to the onset of tumours. In fact N-nitroso compounds (collectively known also as nitrosamines) seem likely to be the major contributors to the chemical origin of various forms of human cancer. Like many other carcinogens, N-nitroso compounds act most effectively when administered in small frequent doses over a long period of time.

The II International Symposium on Nitrite in Meat Products (Tinbergen & Krol, Eds., 1977) established that endogenous (or "in vivo") formation of carcinogenic N-nitroso compounds is probably a more important carcinogenic hazard to man than the presence of exogenous (or preformed) N-nitroso compounds in food. In the meantime, it was also pointed out that cured meat is one of the most important sources of nitrite for "in vivo" formation of volatile carcinogenic N-nitroso compounds.
Hence, given the well-known or potential carcinogenic activity of nitroso compounds it is highly recommended to reduce their formation in food, in order not to add a significant contribution to exposure of such compounds upon reaction of food components with endogenous nitrite.

5.9 Carcinogenicity, Teratogenicity & Mutagenicity of Nitrites, Nitrates & Nitrosamines

There is no definite evidence suggesting that either nitrate or nitrite is carcinogenic in humans. However, several studies have determined that they are not directly carcinogenic, teratogenic or mutagenic in animals (Arbuckle et al., 1988). In the case of nitrite, limited data shows that although it does not behave directly as a carcinogen (unless extremely high doses of both nitrite or nitrosatable precursors are administered), it was found to be mutagenic in microbial systems (Joint FAO/WHO Expert Committee on Food Additives, 1996a, 1996b).

On the other hand, N-nitroso compounds have been found to be clearly carcinogenic in practically all species of animals. Positive results were in fact obtained for approximately 90% of the 300 N-nitroso compounds tested for carcinogenicity in one or more species. Most nitrosamines were also found to be mutagenic while
nitrosamides were shown to be teratogenic in animals. Hence there is a strong evidence pointing at carcinogenicity of N-nitroso compounds in humans (Gouetfongea, 1994; US Assembly of Life Sciences, 1981).

Carcinogenicity of nitrosamines may be enhanced or inhibited. In fact, it was shown, for example, that:

(a) carcinogenicity may be enhanced by agents that promote cell proliferation in the liver;

(b) carcinogenicity may be inhibited by antioxidants such as ascorbic acid and α-tocopherol which are able to block the formation of N-nitroso compounds from reaction of nitrite and nitrosatable substrates.

Hence, diets low in Vitamin C content may create conditions which favour the "in vivo" formation of N-nitroso compounds in humans.

In spite of all this, there is no direct evidence, yet, proving that the current levels of nitrosamines present in the diet are hazardous to human health. However, given the clear correlation between added nitrite levels in foods and the formation of volatile nitrosamines, it is strongly recommended that the formation of N-nitroso compounds should be minimized by, for example, lowering nitrite addition to foods to the minimum possible so as to:
(i) achieve the required preservative effect;

It must be pointed out that nitrosamines were also found to occur in human gastric juice, possibly deriving from nitrites and amines naturally present in the diet. This is why several countries are reducing the legal limit of nitrite used in the curing mixture and enforcing the addition of Vitamin C which inhibits the formation of nitrosamines. Other substances found to be effective in reducing nitrosamine formation are erythorbic (isoascorbic) acid and tocopherol.

In the case of nitrate, there seems to be no clear correlation between added amounts and the formation of volatile nitrosamines and it appears that further information is required about the potential involvement of nitrate in the formation of non-volatile N-nitroso compounds (which may sometimes exceed the level of the volatiles). Such information should be extremely important because although little is known about the occurrence and toxicological properties of such non-volatile nitrosamines, some of them were already found to be mutagenic in nature (US Assembly of Life Sciences, 1981; Walker, 1990).
Finally, considering the varying number of adverse health effects known or suspected to be associated with nitrate, nitrite or \(N\)-nitroso compounds, the true significance of these compounds to human health remains to be determined. Although at present, we cannot quantify the magnitude of the risk involved in the use of such preservatives (and metabolites), the possibilities and chances for hazards do clearly exist (Bruning-Fann & Kaneane, 1993).
6.1 Food Legislation in Malta

The essence of food legislation in Malta is outlined in the Food, Drugs & Drinking Water Act, 1972 (Act No XL of 1972) (Govt. of Malta, 1972) which has ever since been amended and updated by a long list of Amendment Acts including regulations on specific categories of food and food additives. The Food Act is divided into 5 main branches:

- Part 1 - General Provisions
- Part 2 - Water
- Part 3 - Meat
- Part 4 - Milk & Milk Products
- Part 5 - Penal & Other Provisions

The main provisions of the Act, which still express the core principles behind all food regulations in force, may be summarised as follows:

1) It is an offence to sell for human consumption any food to which substances have been added or abstracted or which have been...
processed to render it injurious to health.

2) It is prohibited to sell to the prejudice of the consumer, food not of the nature, substance or quality demanded.

3) It is unlawful to use false or misleading descriptions, and to sell unfit food.

Regulations to control the composition and labelling of food products (and their eventual amendments) in Malta fall within the jurisdiction of the Ministry of Health which acts on the advice of the Food Advisory Committee (FAC) of the Malta Standardisation Authority (MSA). The FAC was set up in 1996 to replace the Food Standards Board of the former Board of Standards.

All regulations related to local legislation on foodstuffs are eventually published officially by the Department of Information and appear in the Government Gazette.

6.2 European Food Legislation (EC Food Law)

The six basic goals for EU legislation (European Commission, 1997) are:
1) to ensure a high level of protection of public health and safety, and of consumer protection;

2) to ensure the free movement of goods within the internal market;

3) to ensure that the legislation is primarily based on scientific evidence and risk assessment;

4) to ensure competitiveness of European industry and enhance its export prospects;

5) to place the primary responsibility for safe food on industry, producers and suppliers, using hazard analysis and critical control points (HACCP) type systems, which must be backed up by effective official control and enforcement;

6) to ensure the legislation is coherent, rational, user friendly and developed in full consultation with all interested parties.

The food law of the European Commission is expressed in a series of Regulations and Directives on foodstuffs, the underlying difference being that:

* **Regulations** must be incorporated into national legislation. These are concerned with primary agricultural produce.

* **Directives** are built into the framework of the individual legislative systems. These are mainly concerned with detailed compositional aspects of manufactured foods.
The EC Food Legislation system is divided into 2 major sections: HORIZONTAL and VERTICAL legislation.

- **HORIZONTAL LEGISLATION**

  Horizontal Legislation (Amaducci, 1996a) covers general regulations on foodstuffs and includes subjects such as:
  
i) labelling;
  ii) prepackaged products;
  iii) materials & articles in contact with foodstuffs;
  iv) additives;
  v) flavours;
  vi) processing aids;
  vii) water for human consumption;
  viii) pesticide residues;
  ix) extraction solvents;
  x) labelling
  xi) nutritional labelling
  xii) hygiene.

- **VERTICAL LEGISLATION**

  Vertical Legislation (Amaducci, 1996b) is concerned with specific products and includes sub-sections such as:
  
i) official control of foodstuffs;
  ii) geographical indications and designations of origin;
  iii) certificates of specific character;
  iv) legislation on specific products (such as cocoa & chocolate products, sugars, honey, fruit juices, preserved milk, natural mineral waters, etc.)
In most cases, legislation lays down the names to be used and gives a definition for each name, specifying also composition, ingredients, additives, conditions of use, manufacturing specifications, special labelling requirements and methods of analysis.

Within the framework of legislation adopted under the 1985 White Paper Programme for processed foodstuffs, priority has in general been given to horizontal measures (which apply to all categories of foodstuffs). Nevertheless, recourse to vertical measures has sometimes been necessary especially in the case of foodstuffs for particular nutritional purposes and quick frozen foodstuffs.

All regulations and directives and any subsequent amendments are published in the Official Journal of the European communities. An important exercise was recently carried out to analyse the current food legislation in the EU countries, with the intention of improving, consolidating and simplifying the Community food law. The outcome of such exercise is contained in the Commission Green Paper, which may therefore be regarded as the new policy paper on Food Law in the European Union (European Commission, 1997).
6.3 Local Regulations on Permitted Food Additives (including Preservatives)

In the process of harmonisation of Maltese legislation with European legislation, food additives started being collectively regulated by the Permitted Food Additives Regulations, 1998, (Govt. of Malta, 1998b) with the exception of colours, sweeteners, flavourings & flour treatment agents.

These regulations, which complement the Additives in Food Regulations, 1994, (Govt. of Malta, 1994a) and repeal the Preservatives in Food Regulations, 1994 (Govt. of Malta 1994b) and its Amendment (Govt. of Malta, 1994c), impose conditions on the addition of a food additive which is define as “a substance not normally consumed as a food in itself and not normally used as a characteristic ingredient of food (whether or not it has a nutritional value)”. The same regulations consider that food additives added intentionally to food (before, during or after processing) may result in the direct or indirect incorporation of the component in the particular food.

In all, there are 23 categories of food additives (one of which being dedicated to Preservatives) classified in regulation 3 (Govt. of Malta, 1998b). The chemical names, E-numbers (Hanssen &
Marsden, 1984) and permitted levels of the individual additives are all tabulated in six schedules as explained hereunder.

♦ **First Schedule**
  - lists those substances generally permitted for use in foodstuffs following the "quantum satis" principle (i.e. no maximum level specified).

♦ **Second Schedule**
  - lists the foodstuffs (e.g. cocoa and chocolate products, fruit juices and nectars, jams, etc.) in which a limited number of additives of the 1st Schedule may be used, specifying such additives and their maximum level permitted (mostly on a "quantum satis" basis).

♦ **Third Schedule**
  - concerns the conditionally permitted preservatives and antioxidants. This schedule is divided into 4 parts:
    ◦ Part A - sorbates, benzoates and p-hydroxybenzoates;
    ◦ Part B - sulphur dioxide & sulphates;
    ◦ Part C - other preservatives, including nitrites & nitrates;
    ◦ Part D - other antioxidants e.g. gallates, BHA & BHT
♦ Fourth Schedule
- deals with other permitted additives (such as phosphoric acid, phosphates, silicates, glutamates, etc.) and their maximum levels of use in foodstuffs ready for human consumption.

♦ Fifth Schedule
- refers to permitted carriers or carrier solvents, and any specific conditions for use in foodstuffs.

♦ Sixth Schedule
- (divided into 4 parts) concerns food additives permitted in Foods for Infants & Young Children.

The original Food Additives Regulations, 1994 (Govt. of Malta, 1994a) which are more generic, specify that a food additive is only approved if it satisfies a set of conditions, namely that:

i) its proposed function cannot be achieved by other means which are economically and technologically practicable;

ii) it presents no hazard to the health of the consumer at the level of use proposed, so far as can be judged on the scientific evidence available;

iii) it does not mislead the consumer.
The second schedule of the same regulations lists the four basic functions of food additives. These are:

(1) **Preservation of Nutritional Quality**

Nutritional quality of food is only accepted to be partly reduced:
- where the food does not constitute a significant item in a normal diet;
- where the additive is required for the production of food intended for consumers under a special diet.

(2) **Special Dietary Needs**

An additive may serve as a necessary ingredient or constituent of foods manufactured for people having special dietary needs.

(3) **Improved Keeping Quality, Stability & Organoleptic Properties**

The additive may be required to enhance the keeping quality or stability of a food or to improve its organoleptic properties, without effecting the nature, substance or quality of food or deceiving the consumer.

(4) **Processing Aids**

Chemicals are also allowed to be added in food to provide aids in manufacture, processing, preparation, treatment, packing, transport or storage of food.
For a food additive to be approved, it has to be specified for which foodstuffs and under which conditions it is to be added. Regulations also stipulate that the lowest possible level of additives have to be used to achieve the desired effect. Furthermore, the acceptable daily intake of the additive has to be taken also into account, keeping in mind the probable contribution of the same substance from other sources. Special considerations on daily intakes have to be taken in the case of consumers under a particular diet.

The law provides also for toxicological evaluation of food additives, taking into account any cumulative, synergistic or potentiating effect of their use and any other health hazards.

Additives permitted for use by the 1998 Regulations on Food Additives are to conform also with purity criteria as laid down by Directive 96/77/EC (European Commission, 1996) of the European Community and any subsequent amendments. Prescribed purity criteria are to be officially checked using the methods of analysis described in MSA 100: 1998 issued by the Malta Standardisation Authority.
6.4 **Local Regulations on Permitted Use of Nitrites & Nitrates**

6.4.1 **Former Regulations (1994)**

The *Preservatives in Food Regulations, 1994* (Govt. of Malta, 1994b) (which have now been surpassed by the *Permitted Food Additives Regulations, 1998*) (Govt. of Malta, 1998b) were enacted to repeal the *Preservatives in Food Regulations, 1977* (Govt. of Malta, 1977). Such regulations concentrated on the use of antimicrobial agents "capable of inhibiting, retarding or arresting the growth of microorganisms or any deterioration of food due to microorganisms or any deterioration of food due to microorganisms, or of masking the evidence of any such deterioration".

Reference to the permitted levels of nitrite & nitrate in meat is made in Part 1 of the 2nd Schedule of these regulations, which concerns specified food which may contain permitted preservatives and the nature and proportion of preservatives in each case.

The following were the categories of meat products mentioned in these regulations and the respective maximum levels of sodium nitrite and sodium nitrate allowed in such type of food.
Table 13

Maximum Permitted Levels on Nitrite & Nitrate in Meat Products
(Source: Government of Malta, 1994b)

<table>
<thead>
<tr>
<th>Meat Product</th>
<th>Maximum Level of $\text{NaNO}_2$ &amp; $\text{NaNO}_3$ (both expressed in mg/kg of NaNO$_2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Cured Meat (including cured meat products) packed in a sterile pack,</td>
<td>150 - total</td>
</tr>
<tr>
<td>whether or not it has been removed from pack.</td>
<td>50 - NaNO$_2$ only</td>
</tr>
<tr>
<td>2) Acidified and/or Fermented Cured Meat Products (including Salami and</td>
<td>400 - total</td>
</tr>
<tr>
<td>similar products) not packed in a sterile pack.</td>
<td>50 - NaNO$_2$ only</td>
</tr>
<tr>
<td>3) Uncooked bacon and ham; cooked bacon &amp; ham that is not, &amp; has not</td>
<td>500 - total</td>
</tr>
<tr>
<td>been, packed in hermetically sealed container.</td>
<td>200 - NaNO$_2$ only</td>
</tr>
<tr>
<td>4) Any cured meat or cured meat product, nor specified above.</td>
<td>250 - total</td>
</tr>
<tr>
<td></td>
<td>150 - NaNO$_2$ only</td>
</tr>
</tbody>
</table>

The local 1994 Regulations, which were based on the U.K. Preservatives in Food Regulations, 1982, (UK Government, 1982) as recommended by the Food Additives & Contaminants Committee, 1978, allowed for the use of equivalent amounts of potassium nitrate and potassium nitrite as alternatives to sodium nitrate and sodium nitrite. The UK Regulations, 1982 have now been superseded by the UK Preservatives in Food Regulations, 1989 (UK Government, 1989), based on an FACC updated report (Food Additives & Contaminants Committee, 1982).
Such regulations distinguished between 4 main types of cured meat products, the highest maximum allowance of total nitrite and nitrate being made for bacon and ham products (whether cooked or uncooked) and the lowest maximum levels being reserved to cured products packed in sterile conditions.

The Working Party on Nitrate & Related Compounds in Food, in its 20th report of the Steering Group on Food Surveillance of the U.K. Ministry of Agriculture, Fisheries & Food (1987) notes that there had been a steady reduction in the maximum observed levels of nitrite and nitrate.

(a) **Nitrate**

The maximum level of nitrate was reduced from 6,000 ppm in 1959 to 1000 ppm in 1972 and around 450 in 1975.

(b) **Nitrite**

Nitrite level underwent a parallel reduction from levels frequently above 100 ppm before 1970 to below 50 ppm in 1973/74.

6.4.2 **Current Regulations (1998)**

The new regulations on the use of nitrites and nitrates in meat products came in force on 1st January 1999 and the maximum permitted levels are recorded in Part C (Other Preservatives) of the...
Third Schedule of the Permitted Food Additives Regulations, 1998 (Govt. of Malta, 1998b).

In the new regulations, conforming with European legislation, both the maximum ingoing amount (i.e. highest level of nitrite/nitrate added during processing) and the maximum residual amount of nitrite and nitrate are specified (i.e. highest level that can be measured in the finished product), as follows:

### Table 14

Maximun Permitted Levels on Nitrite in Meat Products
(Source: Government of Malta, 1998b)

<table>
<thead>
<tr>
<th>Meat Product</th>
<th>Maximum Level of Nitrite i.e. KNO₂ (E249); NaNO₂ (E250)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Indicative Ingoing Amt. (as mg/kg of NaNO₂)</td>
</tr>
<tr>
<td>1) Non-heat treated, cured dried meat products e.g. Parma ham &amp; Italian Salami.</td>
<td>150</td>
</tr>
<tr>
<td>2) Other cured meat products e.g. Danish Salami &amp; canned meat products.</td>
<td>150</td>
</tr>
<tr>
<td>3) Cured bacon.</td>
<td></td>
</tr>
</tbody>
</table>

Determination of Residual Levels of Nitrites & Nitrites in Meat Products Page 122
Table 15

Maximum Permitted Levels on Nitrate in Meat Products

(Source: Government of Malta, 1998b)

<table>
<thead>
<tr>
<th>Meat Product</th>
<th>Maximum Level of Nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>i.e. KNO₃ (E251); NaNO₃ (E252)</td>
</tr>
<tr>
<td></td>
<td>Indicative Ingoing Amt. (as mg/kg of NaNO₃)</td>
</tr>
<tr>
<td>All cured and canned meat products</td>
<td>300</td>
</tr>
</tbody>
</table>

1 Non-heat heated, cured dried meat products (low $a_w$)

Raw cured meat products are usually considered “dried” if they have a low water activity ($< 0.93$) (US Assembly of Life Sciences, 1982). Products include:

a) Scotch, prosciutto, Westphalian, and country ham, dry-cured bacon and dried sausages, all of which may be cold-smoked and not heated appreciably during processing;

b) certain sausages, which may receive some mild heating if smoked (Nitrite Safety Council, 1980; Kramlich et al., 1973).

Many dried meat products are produced with added nitrite (e.g. dried beef, jerky, dry-cured bacon, dry-cured ham and many dried sausages). Preservation of such products at room
temperature relies practically on relatively high salt concentration; and consequently, low water activity (Komarik et al., 1974).

2 **Raw, cured products (high $a_w$)**

Raw cured meats with $a_w > 0.93$ include the form of corned beef that is packaged raw in free pickling solution (Price & Schweigert, 1971) and bacon. Some of them (e.g. bacon) may be subjected to some smoking and mild heating during production (Kramlich et al., 1973).

3 **Cooked, cured products**

According to Price & Schweigert (1971), this is by far the largest category of meat products produced with added nitrite. Such products include:

a) pasteurised products (including hams in casings/cans, frankfurters, bologna, liver sausage, meat loaves, non-specific loaves and some roll products) which are heated to a centre temp. of 65-75°C (US Assembly of Life Sciences, 1982).

b) "shelf-stable" products (such as canned luncheon meats and pre-fried canned bacon (Cerveny, 1980) which are heated to a centre temperature of 95-112°C, which alone is not sufficient to kill spores of *Cl. botulinium* or other
organisms, but may retard their outgrowth in conjunction with other factors, e.g. addition of nitrite.

c) "commercially sterile" products (e.g. corned beef hash, deviled ham, meat spreads and Vienna sausages) which receive thermal processing of at least 2.78 min. at 121°C to eliminate any pathogens and other organisms which usually grow under normal non-refrigerated storage conditions (US Assembly of Life Sciences, 1982).

4 Cured bacon

This is usually classified on its own because:

- it is a raw cured product (generally not being heated sufficiently to become pasteurised), but
- it is more akin to cooked, cured products, with respect to its microbial profile (US Assembly of Life Sciences, 1982).

One must note that levels of nitrite were reduced in some cases (e.g. bacon and heat processed cured/canned products) in a space of 4 years (from 1994 to 1998), while nitrate levels were now established to a separate common maximum permitted amount of 250 mg/kg.
6.5 Other Permitted Preservatives & Regulations on Meat Products

It must be pointed out that nitrites and nitrates are not the only antimicrobial preservatives allowed by the Permitted Food Additives Regulations, 1998, (Govt. of Malta, 1998b) for use in the preparation of meat products. The only other preservatives permitted by current legislation, in meat products, are wood smoke (or liquid solutions of smoke) and sulphur dioxide, which is allowed to be added to a maximum level of 450 ppm in the following products:

i) 'burger meat' - with a minimum vegetable and/or cereal content of 4%;

ii) 'breakfast sausages'.

Former legislation, i.e the Preservatives in Food Regulations, 1994, (Govt. of Malta, 1994b) cited the same maximum permitted level (i.e. 450 ppm) of sulphur dioxide for virtually the same categories of meat products (i.e. similar description), namely:

i) hamburgers or similar products;

ii) sausage or sausage meat.

In the latter case, the 1994 regulations defined the term 'sausage' to exclude any cured meat product which had been acidified or fermented.

Another substance which used to be employed in sausage
meat and in curing (which no longer appears in the permitted list of preservatives of meat products) was boric acid. Two other chemicals which were once considered (in the 40s and 50s) as possible antimicrobials were carbon dioxide and ozone, both of which discourage the growth of surface microorganisms on beef carcasses during prolonged storage at chill temperatures.

Apart from the 1998 regulations covering the use of additives (including preservatives) in food, there are several other generic and specific regulations concerning meat and meat products, which still complement the local Food Act. These are:

♦ Meat Regulations, 1977 (LN 15/77);
♦ Meat Importation Regulations (LN 28/77);
♦ Sausages, Salted Meats & Prepared Meat Regulations, 1977 (LN 156/77);
♦ Corned Beef Regulations, 1981 (LN 100/81);
♦ Luncheon Meat Regulations, 1981 (LN 103/81);
♦ Meat (Amend.) Regulations, 1984 (LN 37/84);
♦ English Type Sausage Meat (Standards) Regulations, 1984 (LN 44/84);
♦ Meat Burgers (Standards) Regulations, 1984 (LN 53/84);
♦ Ground, Minced & Chopped Meat (Standards) Regs, 1984 (LN 59/84);
6.6 **Opinion Expressed by the EU Scientific Committee for Food on the Use of Nitrate & Nitrite in Meat**

In its report on the safety of nitrites and nitrates as food additives (opinion expressed on 19 October 1990) (Bender, 1992) the Scientific Committee for Food of the Commission of the European Communities has pronounced itself on several technological and toxicological aspects of the whole matter before giving its recommendations to the legislators (Commission of the European Communities, 1992).

6.6.1 **Technological Considerations**

The committee found that the level of nitrite to be added to the product depends on the initial level of contamination and has an effect on the general shelf-life (preventing off-flavours). Hence the organoleptic shelf-life should not be set for an unrealistically long period of time as nitrite is very effective against pathogens (including *Clostridium botulinum*) but does not inhibit other spoilage organisms such as *Listeria*, certain *Salmonella* and *Yersinia enterocolitica*.

It was also established that the minimum effective concentration of nitrite with respect to Cl. botulinium depends on a number of variables such as hygienic status, pH, water activity, concentration of salts, etc. According to the EU expert committee (Commission of the European Communities, 1992), if HACCP techniques are employed:
i) the right ingoing amount of nitrite (as sodium nitrite) for many
meat products should be 50-100 mg/kg product;

ii) higher amounts (up to 150 mg/kg) may be required for certain
products (e.g. bacon and ham);

iii) residual levels of nitrite is usually less than 50 mg/kg.

Having realised that certain Member States (of the EU) allow higher maximum levels of nitrite, the Scientific Committee on
Food recommended that production methods must be gradually
improved to reach the point where the low levels could be employed,
whilst achieving the same microbiological safety.

With regards to nitrate, the committee found that if the
curing technique is carried out under strictly controlled conditions,
there seems to be no need for the combined use of nitrate and nitrite.
Therefore, although the nitrate content 'per se' is of no toxicological
concern, nitrate may be reduced (in an uncontrollable way) to nitrite
by means of bacteria, leading to high concentrations of nitrite,
possibly leading to increased formation of undesirable reaction
products (N-nitroso compounds). On the other hand, it was
recognised that nitrate could serve, under certain production
conditions, as a necessary reservoir for nitrite.

Hence, although the careful use of nitrate was not
opposed, the committee recommended changes in production methods in order to reduce and, when feasible, to abandon the combined use of nitrate and nitrite. Preference should be given to addition of nitrite (no nitrate) with salt in order to limit the amount of nitrite added and prevent accidental poisoning through the addition of excessive quantities to food (Commission of the European Communities, 1992).

6.6.2 Toxicological Considerations

The same report specifies that the acute toxic effects of nitrite include relaxation of smooth muscle, vasodilation and lowering of blood pressure, as well as methaemoglobinaemia ($LD_{50} \approx 100-200$ mg/kg body weight).

Considering the results of various studies on toxicity, mutagenicity and carcinogenicity of nitrites and its metabolites (such as N-nitroso compounds) as well as safety limits, the (temporary) Acceptable Daily Intake (ADI) of nitrite was estimated as 0.1 mg/kg bw expressed as sodium nitrite. This ADI is not applicable to infants under 3 months of age (Commission of the European Communities, 1992; JECFA, 1996a).

Nitrate was reported to have a very low acute toxicity, with the only adverse effects deriving from its reduction to nitrite either before ingestion of ‘in vivo’. The ADI of nitrate was established
as 5 mg/kg bw. However, the committee suggested not to allow the use of nitrate as an additive in infant foods since infants are more prone to reduce exogenous nitrate to nitrite and are more sensitive to the acute effects of nitrite (Commission of the European Communities, 1992; JECFA, 1996b).

Three important conclusions reached by the EU Scientific Committee for Food (Commission of the European Communities, 1992), were:

1. the use of nitrate as food additive makes a minor contribution to the total intake of nitrates, the major sources being vegetables and drinking water;

2. the intakes of nitrite and nitrate from food are generally well within the ADIs; the only exceptions are areas of high levels of nitrate in vegetables and drinking water;

3. the use of nitrite and nitrate as food additives should not bring about any direct toxic effects so long as they fall within the levels cited in the same report.

6.6.3. Recommendations

One of the main recommendations made by the committee in view of the carcinogenicity of nitrosamines is the minimization of exposure to such preformed nitrosamines by, for
example lowering levels of added nitrite and nitrate in foods to the minimum required to achieve the necessary preservative effect and ensure microbiological safety.

It was also recommended that further research should be carried out on:

♦ methods of inhibiting nitrosation reactions in foods;
♦ possibility of developing alternative preservatives.


Article 1 of the 1995 Directive stipulates that only the additives which satisfy the requirements laid down by the Scientific Committee for Food are to be used in foodstuffs. The categories of food additives covered by the directive, defined in Article 1, are identical to the ones listed in the local Permitted Food Additives Regulations, 1998 (Govt. of Malta, 1998b). The same applies to maximum levels (including those of nitrate and nitrite in meat products) found in Annexes I-V, the contents of which correspond to information registered in the first 5 schedules of the cited regulations.

Therefore, with regards to the use of nitrite and nitrate in cured meat products, the European Union legislation conforms with maximum levels as tabulated in section 6.4.2.

6.8 U.S. Regulations on Nitrite & Nitrate

In the U.S.A., regulations on the use of nitrate and nitrite may be found in the Code of Federal Regulations, 1998 (CFR) published by the US Department of Agriculture (USDA) in conjunction with the US Food & Drug Administration (1998).

In such regulations, nitrates and nitrites are cited under 2 separate titles, Title 9 and Title 21, as follows:
6.8.1 Use of Nitrites & Nitrates in Red Meat & Poultry

The use of nitrites and nitrates in red meat and poultry is permitted under Title 9 Part 318.7 of the CFR and administered by the Food Safety & Quality Service of the USDA (Houston, 1979). It is noteworthy mentioning that the Federal Meat Inspection Regulations were amended in 1979 to permit meat products traditionally preserved with nitrates (e.g. bacon, corned beef and frankfurters) and/or nitrites to be prepared without nitrites or nitrates and be labelled and sold under the same name as that preserved with the additives, so long as the term "uncured" precedes such name and forms part of the product name.

The US Federal Register (Houston, 1979) stipulates also that labelling of such products should include certain cautionary information such as:

a) the declaration "No Nitrate or Nitrite Added";
b) the statement "Not Preserved - Keep Refrigerated Below 40°F. At All Times".

(unless they have been thermally processed, fermented or pickled to pH 4.6 or less, or dried to a water activity ≤ 0.92).

Products containing an amount of salt sufficient to achieve a brine concentration ≥ 10% are not subject to the above labelling regulations (Houston, 1979).
Furthermore, the same document prohibits the use of nitrates or nitrites in baby, junior and toddler foods.

6.8.2 Use of Nitrite and Nitrate in Red Meat Products & Cured Meat Products

Nitrates (KNO₃ and NaNO₃) are listed as “subject to prior sanctions” for use as sources of nitrate, with or without any nitrite, in the production of red meat products and cured poultry products under Title 21 Part 181.33 of the CFR. Similarly, nitrites (KNO₂ and NaNO₂) are listed as “subject to prior sanctions” for use as colour fixative and preservative agents in the curing of red meat and poultry, with or without nitrate, in Title 181.34 (US Food & Drug Administration, 1998).

6.8.3 Maximum Levels of Nitrites & Nitrates

The maximum levels of nitrites and nitrates to be added to food products (mainly fish and meat products) are listed under the following captions:

(1) Nitrates & Nitrites in Cured Meat/Fish Products

Title 21 Part 172.160 (KNO₃) and Part 172.170 (NaNO₃) concern the use of nitrates while Part 172.175 (NaNO₂) and Part 172.177 (KNO₂) regulate the nitrite addition.
Table 16

Maximum Levels of Nitrates & Nitrites in Cured Meat/Fish Products

<table>
<thead>
<tr>
<th>Salt</th>
<th>Max. Level</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO₃</td>
<td>500 ppm</td>
<td>Preservative and colour fixative in smoked, cured sablefish, smoked salmon, and smoked cured shad</td>
</tr>
<tr>
<td>NaNO₂</td>
<td>200 ppm</td>
<td></td>
</tr>
<tr>
<td>NaNO₃</td>
<td>500 ppm</td>
<td>Preservative and colour fixative in meat-curing preparations for the home curing of meat &amp; meat products (including poultry &amp; wild game)</td>
</tr>
<tr>
<td>NaNO₂</td>
<td>200 ppm</td>
<td></td>
</tr>
</tbody>
</table>

(2) Levels of Nitrite in Processing Smoked Chub
(Title 21: Part 172.177)

Sodium nitrite was also found to act effectively in combination with salt (sodium chloride) by inhibiting the growth and toxin formation from *Clostridium botulinum* type E in the commercial processing of smoked chub. The following are the permitted amounts:

Table 17

Min. & Max. Levels of Nitrite & Salt in Smoked Chub

<table>
<thead>
<tr>
<th>Salt</th>
<th>Min. Level</th>
<th>Max. Level</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>3.5 %</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>NaNO₂</td>
<td>100 ppm</td>
<td>200 ppm</td>
<td>As Preservative (antibotulinal agent) in commercial processing of smoked chub.</td>
</tr>
</tbody>
</table>
(3) **Levels of Other Products**

The same 1981 regulations permit up to 10 mg/kg residual sodium nitrite in smoked tuna as a colour fixative and up to 200 mg/kg residual potassium nitrate as a curing agent (US Food & Drug Administration, 1998).

### 6.9 **International Standards**

Although food regulations may vary from one country to another (if not from one region/state to another) and are entrenched in the various legislative systems on a national basis, there are several international standardising bodies which serve as outstanding points of reference in the preparation and publication of universally accepted standards.

The aim of international standards is to make life simpler and increase reliability and effectiveness of goods and services provided. Such standards are documented agreements containing technical specifications or other precise criteria to be used consistently as rules, guidelines, or definitions of characteristics, to ensure that materials, products, processes and services are fit for their purpose.
International organisations dealing with food standards include:

1. Codex Alimentarius Commission (CAC) of the Joint FAO/WHO Food Standards Programme
2. International Organisation for Standardisation (ISO)
3. International Union of Pure & Applied Chemistry (IUPAC)
4. Association of Official & Analytical Chemists (AOAC)
5. European Committee for Standardisation (CEN)

Such institutions also develop methods of sampling and analysis especially for primary agricultural produce, in conjunction with the various international bodies representing individual commodity interest.

International standardisation has been instrumental in overcoming the so-called “technical barriers to trade” brought about by the existence of non-harmonized standards for similar technologies in different countries or regions. It results from consensus agreements reached between all economic players in a particular industrial sector (e.g. food) - suppliers, users and often governments. It is a known fact that users have more confidence on products and services that conform to International Standards (ISO internet web-sit, 26/5/99).

6.9.1 **European Standards**

The European Committee for Standardisation (CEN) aims
at promoting voluntary technical harmonization in Europe (in conjunction with worldwide bodies and its partners in Europe) to diminish trade barriers, promote safety and allow interoperability of products, systems and services, as well as to promote common technical understanding. The Malta Standardisation Authority (MSA) is affiliated to the CEN and is expected to join it as a full member in the coming years (CEN internet web-site; 26/5/99).

The **European Standards** (ENs) issued by the CEN are established as a general rule because it is important that members' national standards become identical wherever possible. CEN members are obliged to implement European Standards by giving them the status of national standard.

### 6.9.2 **Codex Standards**

Codex Standards are international standards prepared by the Codex Alimentarius Commission set up in 1962 by the Joint FAO/WHO Food Standards Programme. The commission is made up mainly of member nations & associate members of FAO and/or WHO.

The aims of the Joint FAO/WHO Food Standards Programme are (Codex Alimentarius Commission, 1993):

(i) to protect the health of consumers and to ensure fair practices in the food trade;
(ii) to promote coordination of all food standards work undertaken by international governmental and non-governmental organisations;

(iii) to determine priorities and initiate/guide the preparation of draft standards through and with the aid of appropriate organisations;

(iv) to finalise standards and after acceptance by governments, publish them in a Codex Alimentarius (Latin, meaning Food Law or Code) either as regional or world-wide standards.

The Codex Alimentarius is a collection of international food standards adopted by the Commission and presented in a uniform manner. It includes:

- standards for all the principal foods, whether processed or semi-processed or raw;
- provisions in respect of the hygienic and nutritional quality of food, including microbiological norms;
- provisions for food additives, pesticide residues and contaminants;
- provisions for labelling and presentations;
- methods of analysis and sampling;
- provisions of an advisory nature, in the form of codes of practice, guidelines and other recommended measures.

By 1994, the Codex Alimentarius Commission had 146
member countries. It had established 237 commodity food standards, 41 codes of practice covering hygiene and food technology, 185 pesticides had been evaluated leading to the establishment of 3,274 maximum residue limits for pesticides, 760 food additives and 25 contaminants had also been evaluated together with 54 veterinary drugs.

Two important sections of the Codex Alimentarius which deal with the use of nitrite and nitrates in meat products are:

1) **Codex Standards for Processed Meat & Poultry Products**

   *(CAC/VOL X: CODEX STAN 88-89 & 96-98-1981)*

2) **Food Additives**

   *(CAC/VOL XIV-Ed 1)*

### 6.9.1.1 Codex Standards for Processed Meat (& Poultry) Products

The Codex Alimentarius quotes 5 separate standards for Processed Meat & Poultry Products (Codex Alimentarius Commission, 1993). These are:

I. Codex Standard for Corned Beef *(CODEX STAN 88-1981)*

II. Codex Standard for Luncheon Meat *(CODEX STAN 89-1981)*

III. Codex Standard for Cooked Cured Ham *(CODEX STAN 96-1981)*

IV. Codex Std. for Cooked Cured Pork Shoulder *(CODEX STAN 97-1981)*

V. Codex Std. for Cooked Cured Chopped Meat *(CODEX STAN 98-1991)*
The following are the maximum ingoing amounts and maximum residual levels (calculated on the total net content of final product) of nitrite (NaNO₂ and/or KNO₂) in the five categories of meat products cited hereabove.

**Table 18**

**Maximum Permitted Levels on Nitrite in Meat Products**

*Source: Codex Alimentarius Commission, 1993*

<table>
<thead>
<tr>
<th>Meat Product</th>
<th>Maximum Level of Nitrite i.e. KNO₂ (E249); NaNO₂ (E250)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Max. Ingoing Amount (as mg/kg of NaNO₂)</td>
</tr>
<tr>
<td>1) Corned Beef</td>
<td>100</td>
</tr>
<tr>
<td>2) Luncheon Meat</td>
<td>200</td>
</tr>
<tr>
<td>3) Cooked Cured Ham</td>
<td>200</td>
</tr>
<tr>
<td>4) Cooked Cured Pork Shoulder</td>
<td>200</td>
</tr>
<tr>
<td>5) Cooked Cured Chopped Meat</td>
<td>200</td>
</tr>
</tbody>
</table>

In all 5 cases, the Codex Commission recommends the ISO/DIS 2918 method of analysis to determine the level of nitrite in the meat products (ISO, 1975).

**6.9.1.2 Codex Volume on Food Additives**

Volume XIV of the Codex Alimentarius is entirely devoted to the use of additives in foodstuffs. This volume (Codex...
Chapter 6

Alimentarius Commission, 1983) comprises the following 5 parts.

- Part I: Definitions.
- Part II: General Principles for the Use of Food Additives.
- Part III: Principle Relating to the Carry-Over of Food Additives into Food.
- Part V: Food Additives Permitted for Use in Codex Standards.

The Commission specifies that the last section is not to be taken as an exclusive list of food additives.

6.10 Food Labelling Regulations

The legal provisions on labelling of food are currently included in the Labelling & Presentation of Foodstuffs Regulations, 1992 (LN 65/92) (Govt. of Malta, 1992) and its subsequent amendments (LN 201/97 and LN 145/98). The MSA is however in the process of finalising its proposed new set of regulations - The Labelling & Presentation of Foodstuffs Regulations, 1999, (Malta Standardisation Authority, 1999) based on the European Directive on (the approximation of the laws of the Members States relating to the) Labelling, Presentation & Advertising of Foodstuffs (79/112/EEC) and
subsequent amendments (European Commission, 1979).

Directive 79/112/EEC was explicitly designed to constitute a single legislative framework for the compulsory rules on the labelling of foodstuffs. It is based upon the principle of functional labelling. Food labelling regulations aim to provide customers with the essential information as regards the composition of the product, its manufacturer, and its methods of storage and preparation which are necessary to ensure consumer safety and fair competition. Such regulations allow producers and manufacturers to provide any additional formation on the food label, provided this is accurate and does not mislead the consumer (European Commission, 1997).

6.10.1 Nutritional & Organic Labelling

In addition to the main labelling requirements, other regulations are being adopted to govern the provision of additional information by producers or manufacturers on a voluntary basis. In Malta, such regulations came into force on 1st January 1999 and are cited as the Nutrition Labelling Regulations, 1998 (L.N. 247/98) (Govt. of Malta, 1998a). They are only compulsory where a nutrition claim appears on labelling, in presentation or in advertising (except in generic advertising).

Similarly, Council Regulation (EEC) 2092/91 sets out rules
governing the use of the Organic Label on vegetables and vegetable products. The E.U. has also recently proposed rules for the use of the organic label on products of animal origin (European Commission, 1991).

6.10.2 **Label Declaration of Preservatives**

The current regulations on Food Labelling, i.e. Labelling & Presentation of Foodstuffs Regulations, 1992, (Govt. of Malta, 1992) stipulate that food additives must be declared according to their function. In the case of additives serving more than 1 function, these have to be declared according to their main function in the food product.

The same regulations list 16 different categories of additives which must be declared in the list of ingredients. This list is expected to increase further in the new regulations with the addition of new categories of additives such as modified starch, firming agents and humectants. One of the main categories of additives mentioned concerns Preservatives, defined in the Permitted Food Additives Regulations 1998, (Govt. of Malta, 1998b) as “substances which prolong the shelf-life of foodstuffs by protecting them against deterioration caused by microorganisms”. All additives, are to be declared by the category name (in this case, Preservatives), followed by the specific name or E-number or serial number, or both name and
number of the additive. The following are the \textit{E-numbers} of nitrites and nitrates which are commonly used as preservatives in meat products (Hanssen & Marsden, 1984):

- E 249 - potassium nitrite;
- E 250 - sodium nitrite;
- E 251 - sodium nitrate;
- E 252 - potassium nitrate.

Both local and E.U. Labelling Regulations lay down provisions for certain food which may be exempted from certain labelling requirements. However, such food (e.g. not prepacked or prepacked for direct sale) which is not marked or labelled with a list of ingredients 'must' still be marked or labelled with an indication of every type of additive contained in the food, so long as the additive concerned serves the function of:

a) an antioxidant;

b) a colour;

c) a flavouring;

d) a flavour enhancer;

e) a preservative;

f) a sweetener.

In other words, if preservatives such as nitrites and nitrites are present in the product, they must definitely be declared on the food label, no matter how the product is sold (i.e. unpacked, prepacked for direct sale, etc.).
Chapter 7

STANDARD METHODS OF DETERMINATION OF NITRITE & NITRATE IN MEAT PRODUCTS

There are several standard methods for the determination of nitrite and nitrate in foodstuffs. Practically all methods involve at least 3 main steps:

i) extraction of water-soluble salts;

ii) determination of nitrite;

iii) determination of nitrate.

Usher and Telling (1975) published a very interesting critical review of the various techniques employed, with special reference to meat products.

Points discussed in such review included the performance of various clearing agents and errors arising from the presence of other additives e.g. ascorbate, phosphate and sulphite. The article concluded that determination of nitrite could be carried out quite readily and accurately while that of nitrate cannot be regarded as completely satisfactory since it is subject to greater variation and interference.
7.1 Extraction Techniques

Both nitrites and nitrates are completely soluble in water. However, the aqueous extracts from meat products are considered to be relatively unstable due to the influence of other constituents present. Two common interferents are ascorbic acid and isoascorbic acid (also known as erythorbic or araboascorbic acid) or their sodium salts. These substances are added to reduce the amount of nitrite needed for the curing and decrease the likelihood of the formation of carcinogenic nitrosamines.

Several extraction techniques that have been recommended include the use of both hot and cold water. The pH value of the extract is kept higher than 5 in order to minimize any losses of nitrate. Furthermore, Norwitz & Kelliher (1987) found that ascorbic and isoascorbic acids do not react with nitrite to a significant extent in a neutral or alkaline solution. One must however note that both ascorbic and isoascorbic acids are unstable under such (neutral or alkaline) conditions. It should also be added that the pH has to be regulated in subsequent deproteinisation stages to values close to the isoelectric point of the soluble protein (i.e. between pH 5.5 and pH 6.5).

Norwitz & Kelliher (1987) also investigated the effect of heat on the extract during digestion and found that decomposition
does not occur significantly upon heating at 80°C for 2 hours. They proposed a tentative method for the determination of nitrite in cured meats (containing ascorbic or isoascorbic acid) using 0.01M NaOH solution for digestion (carried out at 80°C for 90 minutes).

In general, Carrez Solution, consisting of zinc acetate and potassium forrocyanide (or hexacyanoferrate(III)), is regarded as the most effective clearing agent for general use (e.g. BSI, 1976; ISO, 1975) The AOAC procedure (AOAC, 1995) makes use of hot water digestion followed by clarification with mercuric chloride. This technique was found to be equal to or better than other techniques in a comparative study (Fiddler & Fox, 1978) of 12 methods for use with frankfurters. Norwitz & Kelliher (1987) suggested zinc sulphate as an alternative clarifying agent.

Tsuji et al. (1996) proposed a simple and rapid deproteinising procedure by precipitation of colloid zinc hydroxide formed by adding constant amounts of zinc acetate and sodium hydroxide to alkaline extracts.

Other studies carried out on the interference from ascorbate were those published by De Siena et al. (1981) and Fox et al. (1984) who re-examined recommended extraction procedures in the hope of finding the optimum conditions for minimum interferences.
Chapter 7

STANDARD METHODS OF DETERMINATION OF NITRITE & NITRATE IN MEAT PRODUCTS

7.2 Determination of Nitrite

7.2.1 Spectrophotometric Determination

Standard methods for the determination of nitrite are based on modified versions of the Griess diazotisation procedure in which an azo dye is produced by coupling a diazonium salt with an aromatic amine or phenol. In order to obtain reliable results, one has to take special care in respect of experimental conditions since factors such as pH, temperature, nature and concentration of reagents all affect the final colour intensity (measured by a UV-visible spectrophotometer).

The main variations in such a spectrophotometric determination of nitrite lie in the choice of the diazotisation and coupling reagents. Commonly used diazotisation reagents are:

i) sulphanilic acid;

ii) sulphanilamide; or

iii) 4-nitroaniline.

On the other hand, the coupling agent may be:

i) naphtylamine;

ii) naphtylamine-7-sulphonic acid (known as Cleve's acid); or

iii) N-(1-naphtyl)ethylenediamine (NED).

Naphtylamine is no longer used on grounds of carcinogenicity while NED is usually preferred to Cleve's acid by
various authorities (e.g. BSI, 1976; ISO, 1975) since it reacts more quickly and is less variable in composition.

In a study of possible organic interferences in the spectrophotometric determination of nitrite by the diazotisation-coupling technique, Norwitz and Kelliher (1986) found that many organic substances (e.g. aliphatic and aromatic amines, phenolic compounds and miscellaneous organic compounds e.g. sugars and acids) interfere in the nitrite determination, usually causing low results. They also found that such interference is usually less using 4-nitrosoaniline and NED as the diazotisation-coupling reagent mix, than using a combination of either sulphanilic acid or sulphanilamide with NED. Organic interference is regarded as an important problem because nitrite is frequently determined in the presence of organic materials as in the characterisation of waters, wastes, food and chemical processes.

Other methods, based on the same principles of spectrophotometric determination of nitrite (but using different reagents and azo dyes formed) have been developed by:

- **Eggerger & Honikel (1979)**
  
  This consists of a rapid method for application to cured meats, based on the use of commercially available reagent kits designed for use in water analysis.
• Nakamura & Murata (1979)
  This technique uses a benzene solution of 4,5-
dihydroxycoumarin to produce an unidentified stable reaction
product with an absorbance maximum at 410 nm.

• Amin (1986)
  This method involves a new diazo reaction system based on the
use of 4-aminobenzotrifluoride and coupling with 1-naphtol.
Such reaction was found to compare favourably with other
Griess systems as regards sensitivity and temperature
dependence.

7.2.2 Chromatographic Techniques
  A number of chromatographic techniques have been
developed in an effort to increase sensitivity and limit of detection of
the estimate of nitrite and in some cases, of nitrate. Such procedures
include:

1) Electron Capture GLC
  Methods employed by Funazo et al. (1980), Chikamoto et al.
(1981), Wu et al. (1984, Tanaka et al. (1981, 1983) and Luckas &
Lorenzen (1984) make use of several derivation reactions as a
preliminary step in the use of electron capture detectors in GLC
methods. Results in cured meat were reported to compare
favourably with the colorimetric method.
2) **High Performance Liquid Chromatography (HPLC)**

Noda et al. (1982) and Kunugi et al. (1983) use other derivatives to estimate nitrite and nitrate simultaneously by HPLC. A rapid strong anion exchange HPLC/UV procedure was also developed by Dennis et al. (1990) for determination of nitrate and nitrite in a wide variety of cured meats.

3) **Ion Chromatography**

This technique has been used to estimate a combination of anions (e.g. chloride, phosphate, curing salts). Examples are the procedures developed by Tateo et al. (1982), Jackson et al. (1984) and De Kleijn & Hoven (1984). Another procedure involving the use of Ion Chromatography (with UV absorbance detection) to determine nitrite and nitrate in meat products is that proposed by Siu & Henshall (1988) who claim recoveries of the ions (nitrite and nitrate) greater than 90%. A similar method adopted by Tsuji et al. (1993) also claims excellent recoveries. Other methods have been described by Lippsmeyer et al. (1990) and Murcia et al (1995).

7.2.3 **Other Procedures**

Alternative techniques which have been recently investigated with success involve the use of Chemiluminescence, as described by Fiddler et al. (1984), Capillary Ion Electrophoresis (CIE) as proposed by Marshall & Trener (1996), Continuous Flow (based on
dialysis) as proposed by Beljaars (1994), Flow Injection (FI) as reported by Ferreira et al. (1996) and Enzymes - the Nitrate Reductase method (Hamano et al., 1983; Korkmaz & Cakmakli, 1993).

The CIE technique (a relatively new technique for the analysis of nitrite and nitrate in foods) was found to be a faster, simpler and more reliable alternative method than colorimetric and ion chromatographic methods for the determination of both nitrite and nitrate in a variety of foods. In addition, the CIE technique has excellent resolving power and relatively low operating costs (Swallow & Low, 1994). However, one problem of this method encountered in some applications is poor sensitivity.

The FI method for the simultaneous assay of nitrite, nitrate and chloride in meat products is based on:

i) potentiometric determination of chloride, and

ii) spectrophotometric determination of nitrite (after allowing reduction of nitrate to nitrite in part of the sample).

This method has a high sampling rate (120 determinations per hour, corresponding to 40 samples per hour). This proposed method claims to have a high sensitivity and to give more accurate results than other methods, with relative errors of less than 6% for chloride and nitrite and 2% for nitrate (Ferreira et al., 1996).
7.3 **Determination of Nitrate**

Nitrate can be determined spectrophotometrically via three general reaction systems:

i) nitration of aromatic compounds in sulphuric acid;

ii) oxidation of alkaloids or aromatic amines in sulphuric acid;

iii) reduction reactions to produce ammonia or nitrite.

The first 2 systems which are based on nitration and oxidation were found to give erratic results and hence preference is usually given to the reductive process. However, the AOAC still recommends a procedure (AOAC, 1995) for meat products, based on the nitration of m-xylenol in sulphuric acid, followed by distillation of the nitro-xylenol into sodium hydroxide to yield a solution for colorimetric determination.

**Nitrate may also be determined via other techniques.**

7.3.1 **Electrochemical Methods**

Various electrochemical procedures have been developed to determine nitrite and nitrate in foodstuffs. Such methods include:

i) **Polarographic Methods**

such as the use of differential pulse polarography, reported by Collet (1983);
ii) Flow Injection Systems

such as the one described by Fogg et al. (1983), making use of a glassy carbon electrode as a detector, and that proposed by Ferreira et al. (1996), the latter method having already been dealt with in section 7.2.3.

Electrochemical methods are attractive because electrodes have been found to be suitable for rapid, routine screening applications, especially in cases of use with similar samples. Samples are usually prepared by:

* extraction with water;
* removal of chloride with silver sulphate;
* destruction of nitrite with sulphamic acid.

The nitrate content can then be determined directly by immersion of the probe in the sample extract.

Application of electrodes to foods have been reviewed in several studies such as those conducted by Pfeiffer & Smith (1975), Liedtke & Meloan (1976) and Henshall et al. (1977). The latter compared 4 different methods of nitrate determination in processed foods and concluded that two of them, namely:

i) the ion-selective method; and

ii) the AOAC procedure involving nitration of m-xylenol,

were not suitable for general use.
7.3.2 Reduction Systems

Henshall et al. (1977) recommended the Follett & Ratcliff (1963) method of nitrate determination, as modified by Evans (1972) in order to eliminate interference by ascorbate. In this method, nitrate is determined by reduction to nitrite using a cadmium column, and subsequent development of an azo dye (using composite diazotisation-coupling reagents) which can be determined spectrophotometrically.

This Cadmium Reduction technique has also been recommended by BSI (1976) and ISO (1975) for nitrate determinations in cheese and meat products. Other reduction techniques involve the enzymatic reduction of water-soluble nitrate to nitrite by nitrate reductase (Hamano et al., 1983 and Korkmaz & Cakmakli, 1993).

7.3.3 Automated Reaction Systems

Henshall et al. (1977) also proposed an automated version of a method, originally developed by Bloomfield et al. (1965). This method involves the quantitative interference of nitrate in a reaction between α-furil-dioxime and rhenium in the presence of tin(II) chloride. Although such reaction system has been found to require a tedious procedure, it was found to be very precise and convenient when automated and applied for large numbers of samples.
7.3.4 Chromatographic Procedures

As in the case of nitrite, nitrate can also be determined by a number of methods involving the use of GLC and HPLC.

* GLC methods were discussed by a number of authors such as Tanaka et al. (1982) and Ross & Hotckiss (1985);

* HPLC methods were dealt with by other authors such as Schmidt & Schwedt (1984), Hunt & Seymour (1985) and Wootton et al (1985) with the latter working specifically on nitrate and nitrite levels in cured meats.

7.4 Preparation of Sample

Every sample of meat product analysed in this project was prepared using the AOAC procedure (AOAC Official Method 983.18, 1995). A representative sample of the product (the entire contents in the case of canned meat or frozen products) was each time precut to a maximum size of about 5 cm and homogenised in a food processor (variable speed 0-21000 revs/min) for a total time of 2 minutes (at a speed of 1725 revs/min).
Samples greater than 100g were used to prevent loss of moisture during preparation and subsequent handling. All samples taken were analysed immediately. In the case of frozen products, the material was first defrosted to room temperature. Sausage meat had to be removed from casings (i.e. deskinned) before being passed through the food processor.

7.5 Preparation of Meat Extract

The meat extract was prepared according to a standard procedure (Kirk & Sawyer, 1997) based on maceration of a homogenised sample with a borax solution, followed by digestion in hot water at 70°C for about 30 minutes. The mixture is then cooled to room temperature and clarified using Carrez solution (i.e. a mixture of zinc acetate and potassium ferrocyanide) generally regarded as one of the best clearing agents for the precipitation of meat proteins. The mixture is subsequently checked for pH (if necessary adjusting it to pH 8.3) and filtered through (nitrate and nitrite-free) fluted filter paper, collecting the aqueous extract in a 200 mL volumetric flask and topping it up to mark with distilled water.
An alternative method developed by Norwitz & Kelliher (1987) and modified by Binstok et al (1996) was also tried on a meat reference material and seemed to yield better overall results. This method involves dispersion of the homogenised sample with 40 g of sand (to increase the surface area), digestion with 0.01M NaOH at 80°C for 90 minutes, and clarification with 10% zinc sulphate solution, followed by filtration with nitrate-&-nitrite-free filter paper. This second procedure adopts the same diazotisation-coupling technique (i.e. using sulphanilamide + NED in HCl), doubling the concentration of NED (coupling agent) and measuring the absorbance of the azo dye at 542 nm (instead of 538 nm).

### 7.6 Spectrophotometric Determination of Nitrite by the Diazotisation-Coupling Technique

*Modified Griess-Illosvay Reaction*

The method chosen in this project for the determination of nitrite is one of the modified versions of the classical Griess diazotisation reaction (Griess, 1879; Illosvay, 1889) in which a red azo dye is formed by coupling a diazonium salt (formed by reacting nitrite with sulphanilamide) with the aromatic amine: N-1-(naphtyl)-ethylenediamine (NED) as shown in figure 9.
Figure 9

Diazotisation and coupling reactions in nitrite determination
NED was preferred to the other coupling agents mentioned in similar diazotisation-coupling methods on the basis of safety (e.g. 1-naphtylamine is carcinogenic), reaction kinetics (dye forms almost instantaneously) and chemical stability (NED being less variable in composition than Cleve's acid, i.e. 1-naphtylamine-7-sulphonic acid).

A known volume of the meat extract is pipetted in a one-mark volumetric flask diluted with water and reacted with sulphanilamide in the presence of hydrochloric acid, to produce the diazo compound. This reaction is followed by the addition of the coupling agent (i.e. NED) which produces the expected red azo dye which absorbs light at a wavelength of 538 nm. The amount of azo dye produced is directly proportional to the level of nitrite present (and hence to the concentration of nitrous acid in the extract).

A calibration curve has to be plotted every time a new set of samples is being analysed by preparing a series of diluted standard sodium nitrite solutions containing 0-100 μg NaN02. These solutions are also reacted with the same amount of sulphanilamide and coupling agent, and the intensity of the absorbance is measured spectrophotometrically at the same wavelength (i.e. 538 nm). The calibration curve is then used to determine the amount of nitrite present in the same extract.
A mean value of nitrite (expressed as mg/kg of sodium nitrite) is finally calculated from 6 determinations of the same homogenised product.

7.7 Determination of Nitrate by the Cadmium Reduction Method
(Modified Follett & Ratcliff Technique)

Given the toxicity of certain reagents (e.g. brucine), the unreliability of others in the presence of carbohydrates and other organic matter (e.g. phenoldisulphonic acid), poor colour stability, poor sensitivity and time-consumption of various nitration and oxidation techniques, the Cadmium Reduction procedure originally proposed by Grau & Mirna (1957), developed by Follett & Ratcliff (1963) and further improved and modified by Evans (1972) still enjoys the recommendation of various organisations such as BSI, ISO and EC.

Other methods, including the AOAC xylenol method (which requires the elimination of chloride and nitrite) are even more laborious and time-consuming and may prove erratic due to interference, mainly from organic matter. The same applies to gasometric methods and techniques depending upon the reduction of nitrate to ammonia.
The modified Follett & Ratcliff method involves passing a given volume of the same meat extract (prepared for nitrite determination) through a glass column packed with metallic cadmium. The nitrate is reduced to nitrite which is then collected and reacted with the same diazotisation-coupling reagents used for nitrite determination to produce the red azo dye measured photometrically at the same wavelength (i.e. $\lambda = 538$ nm). The total amount of nitrite (determined after reduction) less the original quantity determined (prior to the reduction step) is equivalent to the nitrate ion concentration.

In spite of the fact that such method has not yet been automated and may create several problems (related with flow rate, packing, oxidation of metal and routine laborious procedures to check the reducing capacity of every column), it is still regarded as one of the most suitable methods for the analysis of nitrate in cured meat products.
The residual amounts of preservatives (nitrite and nitrate) in the samples provided were determined using standard procedures currently in use in many countries and recommended by various international organisations.

8.1 Principle

8.1.1 Determination of Nitrite

Test portions were extracted from the homogenised samples by hot water digestion (following pre-treatment with borax solution), precipitation of proteins by adding solutions of potassium hexacyanoferrate(III) and zinc acetate, and filtration using nitrite-free fluted filter paper. Each filtrate was then treated with sulphanilamide and N-(1-naphtyl)ethylenediamine dihydrochloride (NED) in the presence of hydrochloric acid, and the resulting red complex was finally measured spectrophotometrically at a wavelength of 538 nm.

8.1.2 Determination of Nitrate

The same test portions obtained for nitrite determination were passed through separate cadmium columns to reduce any
The nitrate present in solution to nitrite. The nitrite collected from each column was then diazotised with sulphanilamide and coupled with NED to produce the same reddish purple azo dye, measured spectrophotometrically at $\lambda = 538$ nm.

The nitrate content could then be determined by deducting the nitrite level (found in the same extract) from the total nitrite concentration (after reduction), and multiplying by a factor representing the ratio of the relative formula masses of sodium nitrite and sodium nitrate.

8.2 **Apparatus**

All glassware used was regularly and thoroughly washed and rinsed in distilled (preferably deionised) water to ensure that it was free from any nitrates and nitrites. The usual laboratory equipment was used, in particular:

1. **Food Processor**
   
   A Sirman food processor (Model C4 W) with variable speed (0-2100 rpm), 3.5L capacity and fitted with a magnetic and mechanical microswitch (to stop machine when lid is removed). The processor was equipped with the electrical and
mechanical safety devices that are available during functioning, cleaning and maintenance.

(2) **Analytical Balance.**

(3) **One-mark volumetric flasks**
   - capacities 100mL, 200mL and 500mL
   (class B, complying with requirements of ISO 1042 or BS 1792)

(4) **One-mark pipettes.**
   - capacities 2, 5, 10 and 20 mL
   (class A, complying with requirements of ISO 648 or BS 1583)

(5) **Fluted filter paper.**
   - 15 cm diameter (free of nitrate and nitrite)*

(6) **Conical flasks.**
   - capacity 300 mL

(7) **Electromagnetic stirrer/hot-plate.**

(8) **Spectrophotometer**
   - fitted with cells of 1 cm optical path length, and suitable for measurements at wavelengths 538 to 542 nm.

(9) **Stirring rods**

(10) **Spatulas.**
(11) Boiling water-bath.

(12) Reduction Columns

- made of glass, as per diagram (Figure 9).

(may be fitted with flexible connection between bottom of column and exit capillary tube to regulate position of outlet and hence flow rate).

* Test for Filter Paper (AOAC, 1995)

The filter paper were tested for nitrite and nitrate contamination by analysing 3-4 sheets, at random, throughout box. About 40 mL of water were filtered through each sheet, adding 4 mL sulphanilamide, mixing and allowing to stand for 5 minutes before adding 4 mL NED reagent, mixing and waiting for 15 minutes. The same procedure was repeated, passing filtrate through the reduction column and adding diazotisation-coupling reagents. Had any sheets been positive, the entire box would have been discarded.

8.3 Reagents & Solutions

All reagents used were of analytical quality. They were all stored in well-stoppered glass bottles. Nitrite and NED solutions were stored in refrigerated conditions. Distilled water produced from the same distiller, was used throughout the entire work.
Apparatus for Cadmium Reduction Method for Nitrate Determination
All dimensions in mm.
(Source: Kirk & Sawyer, 1997)
Figure 11

Battery of Cadmium Reduction Columns for Nitrate Reduction

Determination of Residual Levels of Nitrites & Nitrates in Meat Products
Fig 12: Close-up of 2 Cadmium Reduction Columns
8.3.1 Nitrite Determination

8.3.1.1 Solutions for Precipitation of Proteins

1) 106g of potassium hexacyanoferrate (III) trihydrate, $K_4Fe(CN)_6\cdot3H_2O$, were dissolved in water & diluted to 1000 mL.

2) 220g of zinc acetate dihydrate, $Zn(CH_3COO)_2\cdot2H_2O$, were dissolved in water adding 30mL of glacial acetic acid and diluting to 1 litre.

3) A saturated borax solution was prepared by dissolving 50g of disodium tetraborate decahydrate, $Na_2B_4O_7\cdot10H_2O$, in 1 litre of tepid water (on a hot-plate/stirrer) and cooling to room temperature.

8.3.1.2 Solutions for Diazotisation-Coupling Reaction (Colour Development)

4) 2g of sulphanilamide, $NH_2C_6H_4SO_2NH_2$, were dissolved in 800 mL of warm water; the solution was then cooled, adding 100 mL of conc. HCl whilst stirring and diluting to 1 litre with water.

5) NED Solution I

0.25g of N-(1-naphtyl)ethylenediamine dihydrochloride (NED), $C_{10}H_7NHCH_2CH_2NH_2\cdot2HCl$, were dissolved in water and diluted to 250 mL. Solution was then stirred in a well-stoppered brown bottle and kept in a refrigerator, being renewed every week.
6) 445 mL concentrated hydrochloric acid, HCl, were added to water and diluted to 1 litre (conc. = 5.42M).

8.3.1.3 **Standard Sodium Nitrite Solutions (Calibration Curve)**

7) **Stock Solution of NaNO₂ (Renewed weekly)**

1.000 g sodium nitrite, NaNO₂, was dissolved in water and diluted to 100 mL. The solution was stored in a refrigerator and renewed weekly.

8) **Working Solutions of NaNO₂ (Prepared daily)**

5 mL of stock solution were diluted to 1 litre with water. 5, 10, 15 and 20 mL of this solution were then diluted to 100 mL with water. The resulting solutions contained 2.5μg, 5.0μg, 7.5μg and 10.0μg of NaNO₂ per millilitre.

8.3.2 **Nitrate Determination**

The same solutions 1-6 used for nitrite determination, were also used for the nitrate assay. Additional reagents that were required included:

9) **Ammonia Buffer Solution (pH 9.6-9.7)**

This was prepared by diluting 20 mL of concentrated hydrochloric acid with 500 mL distilled water, adding 10 g of disodium dihydrogen ethylene-diamine-NNN'N'-tetraacetate
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dihydrate, \([\text{CH}_2\text{N(CH}_2\text{COOH})\text{CH}_2\text{COONa}]_2\cdot\text{2H}_2\text{O}\) and 55 mL concentrated ammonia solution. The solution was diluted to 1 litre and checked for pH (9.6-9.7).

10) Cadmium coarse powder “for reductors” (size: 0.3-1.6 mm).

11) Dilute Hydrochloric Acid (about 0.1M)

8 mL of concentrated HCl were diluted to 1 litre with water.

12) Standard Potassium Nitrate Solution

This was prepared by dissolving 1.456g potassium nitrate, KNO₃, in water, and diluting to 1 litre.

8.3.3 Other Reagents/Solutions Used

13) Activated charcoal (for decolorisation of filtrates).

14) Sand GPR.

15) 10% w/v zinc sulphate solution, prepared by dissolving 10g of zinc sulphate heptahydrate, \(\text{ZnSO}_4\cdot\text{7H}_2\text{O}\), in 100 mL of water.

16) 0.01M NaOH solution.

17) 1M NaOH solution.

18) 4M HCl solution.
19) **NED Solution II (Double Conc.)**

0.5g of NED were dissolved in 250 mL water. The solution was stored in a brown bottle and refrigerated.

20) **Concentrated phosphoric acid, H₃PO₄.**

### 8.4 Nitrite Calibration Curve

A calibration curve was prepared each time a fresh batch of samples were to be analysed. This was done by using water and 10 mL of each of the working solutions of sodium nitrite (i.e. diluted standard solutions of NaNO₂), in separate 100 mL volumetric flasks, the new solutions containing 0, 25, 50, 75 and 100μg of sodium nitrite. Water was added to each flask to increase the volume to about 60 mL. 10 mL of sulphanilamide (reagent 4) were then added, followed by 6 mL of hydrochloric acid (reagent 6). The solution was mixed and left for about 5 minutes in the dark. This was followed by the addition of 2 mL of the coupling agent - NED (reagent 5). The resulting solution was mixed again and left for 3 to 10 minutes at room temperature in the dark to allow the development of the red-purple azo dye. The solution was finally diluted to the 100mL mark with water and its absorbance was measured by means of a spectrophotometer at 538 nm in a 1 cm cell.
The graph is drawn by plotting the values for absorbance of each solution against the concentration of nitrite (covering range 0-100μg). From the calibration curve, the mass of NaNO₂ in μg (m₁) equivalent to the absorbance of the test sample is read off.

♦ **NB:** If the sample reading exceeds absorbance of the top standard solution, the nitrite concentration is found from the gradient of the curve or by measuring the absorbance of a smaller aliquot of the test solution.

### 8.5 Preparation of Test Sample

**AOAC Method 973.31 (AOAC, 1995)**

A representative sample of the product (about 200g or a whole batch) was chopped to small pieces (size < 5 cm) and homogenised in a food processor in 30 second spans, for a total duration of 2 minutes, wiping down three times the inner side wall and bottom of the bowl with a plastic spatula to transfer material gathered to the body of the sample.

Special care was taken with certain meat products to assure uniform distribution of fat and connective tissue. These should be reincorporated into sample by means of a spatula at each wipe-down interval. Should the sample consolidate as a ball above the blades, the food processor has to be stopped for a while such that the
sample is pressed to the bottom of the bowl before resuming homogenisation.

8.6 Preparation of Meat Extract
(Precipitation of Protein)

8.6.1 Standard Extraction Method (ISO, 1975; Kirk & Sawyer, 1997)

An amount $m_o$ (10-20g) of homogenised sample was weighed out to the nearest mg into a 300 mL conical flask. The sample was macerated with 5 mL borax solution (reagent 3) and 70 mL of hot distilled water (with temperature not lower than 70°C). 75 ml of hot water were added and the resulting mixture was heated on a water-bath for 30 minutes, with occasional stirring.

The contents of the flask were then allowed to cool and clarified by adding 2-5 mL of potassium hexacyanoferrate (III) (reagent 1) and 2-5 mL of zinc acetate (reagent 2) followed by mixing. The pH of the supernatant was checked/adjusted to pH 8.3; pH was regulated by dropwise addition of 1M NaOH solution or 4M HCl. The mixture was allowed to stand for about 30 mins. and filtered through a fluted filter paper (certified free from nitrite and nitrate), collecting the clear filtrate in a 200mL volumetric flask. When the filtrate was coloured due to the presence of natural pigments and/or artificial dyestuffs in solution, it had to be decolourised by the addition of 0.5g activated
charcoal, followed by a second filtration. When the colour persisted, a second dose of charcoal was added, followed by a further filtration.

The filtrate (meat extract) was finally diluted to mark (200 mL) with distilled water. Known volumes of the same filtrate were taken for the determination of nitrite and nitrate, as described in sections 8.7.1 (nitrite) and 8.8.4 (nitrate).

8.6.2 Modified Norwitz-Kelliher Technique (Binstok et al. 1995)

Binstok et al. (1995) developed an alternative extraction technique which is claimed to result in a higher recovery of nitrites (about 93%) showing also a high precision.

This method involves the dispersion of about 10g of homogenised sample with 40g sand (to increase surface area) and digestion with 150 mL of 0.01M NaOH in a hot water-bath (80°C for 90 minutes), stirring occasionally.

The mixture is clarified by adding 5mL of 10% w/v ZnSO₄ (zinc sulphate heptahydrate) to the hot solution (T>80°C) whilst swirling. The mixture is then cooled to room temperature and checked for pH (≈7.5) prior to filtration through nitrite-free filter paper.
The filtrate is then assayed spectrophotometrically for nitrite and nitrate as described in sections 8.7 and 8.8, the only differences being:

(a) the use of double the concentration of coupling reagent NED (i.e. reagent 19);
(b) measurement of absorbance at $\lambda = 542$ nm (not at 538 nm).

### 8.7 Nitrite Determination

*(Modified Griess-Ilosvay Reaction)*

#### 8.7.1 Colorimetric Reaction

$V_1$ mL (about 40 mL) of filtrate (meat extract diluted to 200 mL) were pipetted into a 100 mL one-mark volumetric flask and distilled water was added to obtain a volume of about 60 mL. 10 mL of sulphanilamide (reagent 4) were then added, followed by 6 mL of hydrochloric acid (reagent 6). The solution was mixed thoroughly and left for 5 minutes in the dark (to allow the formation of the diazonium salt).

2 mL of the coupling reagent NED (reagent 5) were also added; the solution was mixed and left again for 3-10 minutes at room temperature in the dark (to allow for the formation of the red-purple azo dye).

The resultant solution was finally diluted to the 100 mL
mark with distilled water and the absorbance measured at a wavelength of 538 nm in a 1cm cell, by means of a suitable spectrophotometer.

8.7.2 Calculation

The nitrite content, N, expressed in mg/kg or ppm of sodium nitrite, could be calculated using the formula:

\[ N = \frac{200 m_1}{m_0 V_1} \]

where:
- \( m_0 \) = mass in g. of test portion;
- \( m_1 \) = value given by the calibration curve for the mass of sodium nitrite in \( \mu g \), corresponding to the absorbance of the test solution;
- \( V_1 \) = volume in mL of the aliquot portion of the filtrate taken for the test.

8.8 Nitrate Determination
(Modified Follett & Ratcliff Method)

8.8.1 Preparation of Cadmium Column

About 100g of cadmium coarse powder (size: 0.3-1.6 mm) were blended with 400 mL of 0.1M HCl, decanted and washed twice using about 1 litre of distilled water. The cadmium was then washed into the glass column shown in diagram (Figure 9) until a column of metal approximately 170 mm long was obtained. The excess water
was drained, taking care not to allow the surface of the column to become exposed to air (i.e. avoiding atmospheric oxidation of cadmium). Any inclusions of the gas were removed with a fine plastic knitting needle and gentle tapping on the glass column. An important parameter for this method is the flow rate for the column which was regulated not to exceed 3ml/min (to allow maximum reduction of any nitrate present in the extract).

Alternatively, the glass column could have been packed with spongy metallic cadmium prepared by placing 5 zinc rods (15 cm length x 5 cm diameter) into 1 litre of 30% w/v cadmium sulphate solution in a large beaker, removing the metallic cadmium formed every 1-2 hours, decanting and washing it twice with 1 litre distilled water. The cadmium formed has to be blended with 0.1 HCl and washed again with distilled water before packing it inside the glass column.

It is convenient to set up a battery of several (e.g. six) columns for routine analysis and multiple determinations.

8.8.2 Pre-treatment of Cadmium Column

Prior to every nitrate determination, the column is washed successively with 25 mL of dilute hydrochloric acid (reagent 11), 50 mL of distilled water, and finally with 25 mL of the ammonia buffer solution (reagent 9), diluted 1:9 with distilled water. Care was again taken to prevent the column from running dry.
8.8.3 **Checking the Reducing Capacity of the Cadmium Column**

The reducing capacity of the column was regularly checked as follows. 20 mL of potassium nitrate standard solution (reagent 12) and 5 mL of the ammonia buffer solution (reagent 9) were pipetted into the reservoir on the top of the column. The effluent was collected in a 100 mL one-mark volumetric flask. When the reservoir was almost empty, it was washed with about 15 mL of 1+9 dilution of the buffer (reagent 9) and allowed to drain. This was repeated with a second portion (15 mL) of the diluted buffer, and again was allowed to drain.

The reservoir was then filled with the diluted buffer and the effluent was collected until the 100 mL flask was almost to the mark. 10 mL of the solution were then pipetted into a 100 mL flask and made to mark. The nitrite content was finally determined by the colorimetric method described earlier.

**NB** If the nitrite content of the effluent is below 0.90 μg of NaNO₂ per ml (i.e. 90% of the theoretical value), the cadmium column should be rejected and prepared afresh.

8.8.4 **Reduction of Nitrate to Nitrite**

The cadmium column was again conditioned by treatment with dilute hydrochloric acid, water and diluted buffer (as described in section 8.8.2). 20 mL of the sample extract (deproteinised prior to nitrite determination) were pipetted simultaneously with 5 mL of the
buffer solution. The effluent was collected and the column washed with the dilute buffer solution as described in the reduction check method (section 8.8.3).

8.8.5 **Colorimetric Reaction**

The effluent was made up to 100 mL, pipetting an aliquot, $V_2$ of not more than 25 mL (e.g. $V_2 = 20$ mL) into a 100 mL one-mark flask. The sodium nitrite content was then determined colorimetrically as described in section 8.7.1 (i.e. by forming a diazonium salt with sulphanilamide in the presence of hydrochloric acid and then by coupling with NED solution to form the red azo dye absorbing at $\lambda = 538$ nm).

8.8.6 **Calculation**

The nitrate content of the sample $S$, expressed in mg/kg (or ppm) of sodium nitrate, was finally determined using the formula:

$$S = 1.203 \left(\frac{1000 m_1}{m_0 V_2}\right) - N$$

*where:*

- $m_0$ = mass in g. of test portion;
- $m_1$ = value given by the calibration curve for the mass of sodium nitrite in $\mu$g, corresponding to the absorbance of the test solution;
- $N$ = nitrite content of the sample in mg/kg NaNO₂ as determined in section 8.7.2
- $V_2$ = volume in mL of the aliquot portion of the filtrate taken for the colorimetric measurement.
Although in the curing industry, results used to be conventionally expressed as sodium nitrite for the Nitrite Determination and as potassium nitrate for the Nitrate Determination, both the Permitted Food Additives Regulations, 1998 (Govt. of Malta, 1998b) and the EU Directive 95/2/EC on Food Additives (European Commission, 1995) (other than colours & sweeteners) express maximum limits of such preservatives in terms of the sodium salts.

8.9 **Checking Percentage Recovery of Nitrite & Nitrate**

The percentage recovery of nitrites and nitrates could be measured by assaying a Control Mixture and Reference Test Materials (containing known amounts of NaNO₂ and NaNO₃) prepared as follows (Binstok et al., 1996).

8.9.1 **Control Mixture (Meat Blank)**

A sample of beef (weighing about 90g, and preferably taken from the "biceps femoris") was trimmed to remove any visible fat and precut to maximum dimensions of 5 cm. The meat was transferred to the food processor bowl, including any separated liquid, and processed (at a speed of c. 1725 rpm) for about 2 minutes in 30-second spans, wiping down the inner side wall of the bowl with...
a plastic scraper in the intervals. Special care was again taken to secure uniform distribution of fat and connective tissue in the sample.

8.9.2 **Nitrite Reference Material**

10 mL of an aqueous solution containing 0.008g of sodium nitrite (i.e. 0.8g nitrite dissolved in 1000 mL water) were added to a second sample of beef, previously weighed (about 90g) and prepared in an analogous way as described in section 8.9.1. The sample was then thoroughly mixed and homogenised in the food processor, adjusting the pH of meat slurry to 5.0 by the dropwise addition of phosphoric acid.

8.9.3 **Nitrate Reference Material**

A third sample (about 90g) was prepared in a similar manner to section 8.9.2 but replacing the sodium nitrite by approximately the same amount of sodium nitrate (i.e. adding 10 mL of solution containing 0.008g NaNO₃).

8.9.4 **Combined Nitrite & Nitrate Reference Material**

A slightly bigger sample (weighing approximately 150g) was prepared using the same technique, this time adding 10 mL of an aqueous solution containing:
(i) 0.004g sodium nitrite (0.4g in 1000 mL);
(ii) 0.028g sodium nitrate (2.8g in 1000 mL).

All reference materials have to be analysed immediately after being prepared, using the same technique adopted in the nitrite and nitrate determination of product samples. The control mixture (meat blank) was analysed to test the interference to the technique being used, due to meat and other reagents present.
9.1 Objectives & Applicable Regulations

The main objective of this survey was to determine (using standard procedures) the levels of nitrite and nitrate preservatives in cured meats which are currently available on the local market. The survey also provided the opportunity to check whether any products locally consumed exceeded the maximum permitted levels of such additives at the time the samples were taken for analysis.

This was not the only survey ever carried out locally on nitrite and nitrate in food. A previous survey of nitrite levels in foodstuffs (mainly cured meat products) was undertaken in 1996 by the Public Health Laboratories of the Health Division (Public Health Labs., Malta, 1996). No samples from the 64 products analysed in that survey were reported to contain levels of nitrite higher than 50 mg/kg. Unfortunately, there is no indication of the levels of nitrate in these samples and it cannot be determined whether any of them exceeded the combined statutory limit in force at that time. Two further surveys carried out by the same Public Health Labs. in 1998
and 1999 focussed on the nitrite content on Maltese-type sausages (Public Health Labs., Malta, 1998 & 1999). Most of the 33 samples analysed in 1998 and the 122 products analysed in 1999 had less than 5 mg/kg of nitrite. However, there were at least 3 cases (1 in 1998, and 2 in 1999) which, surprisingly enough for such type of ("uncured") product, contained about 40 mg/kg of nitrite and one product (in 1999 survey) which was found to have 98.7 mg/kg of the same preservative. Once again, it is very difficult to make any legal implications on the cases in question since no nitrate values are available.

This particular survey is therefore probably the first attempt in recent years to assess the amount of both preservatives in meat products purchased in Malta. Although the findings from this survey may throw some light on the residual levels of nitrite and nitrate found in both local and imported meat products, it must be said that the study was carried out in the year (1999) when the new regulations - Permitted Food Additives Regulations, 1998 (Govt. of Malta, 1998b) - came in force. Such regulations (now conforming with EU Regulations) limit the maximum residual concentration of NITRITE in cured bacon to 175 mg/kg and to 100 mg/kg in other cured meat products (in both cases as sodium nitrite). On the other hand, the maximum permitted residual concentration of NITRATE in all cured meat products now stands at 250 mg/kg (as sodium nitrate).
Hence, having been conducted early in 1999, with most products analysed being produced in the previous year (1998), the maximum levels of nitrite and nitrate applicable for practically all cases are those specified in the Preservatives in Food Regulations, 1994 (Govt. of Malta, 1994b). These 1994 regulations limited the combined amount of nitrite and nitrate in cured meats to between 150-500 mg/kg (the higher figure applying for uncooked or hermetically sealed bacon and ham), depending upon the type of processing and packaging used. The same regulations specified that the maximum allowance of residual nitrite in cured meat products was 50-200 mg/kg (as sodium nitrite), the higher level being once again conceded in the case of the bacon and ham products previously cited.

Had a similar survey been carried out by the Public Health authorities during such a transitional period of legislation, information such as date of manufacture/processing and "best before" date would have been crucial in determining with certainty which set of regulations would have applied for every sample analysed.
9.2 **Methodology**

Samples taken for this particular survey were obtained from supermarkets, minimarkets and other outlets in various parts of the island (mainly at Zurrieq, Paola, Msida and Valletta). In all, a total of 50 samples, 25 local and 25 imported products were purchased at random and analysed between January and May 1999.

Although no specific sampling programme was followed, the products analysed were chosen to reflect the 3 main categories of meat products commonly purchased from supermarket/minimarket shelves and delicatessen counters, or from local butchers' shops. Products were therefore grouped as follows:

- **Category A** - Canned Shelf-Stable Products;
- **Category B** - Refrigerated Products;
- **Category C** - Frozen Products.

All samples purchased for the survey were transported to the laboratory and processed immediately. Each product was first homogenised in a food processor and then divided into 6 portions, with each portion being macerated with borax solution and extracted with water at 70°C before being clarified with potassium hexacyanoferrate (III) and zinc acetate, filtered and analysed spectrophotometrically (and separately) for nitrite (using the *modified Griess-*...
Illosvay technique) and nitrate (using the Cadmium Reduction method, followed by the modified Griess-Illosvay reaction).

For each set of determinations, a nitrite calibration curve was prepared to minimize any errors from reagents/solutions and instrumentation.

With the exception of 3 results (for which sample size was n=5), the mean was calculated from a set of six determinations (n=6) and reported to one decimal place as mg/kg of sodium nitrite or sodium nitrate. Hence the total number of determinations carried out during the entire survey was 594, divided as follows:

- 297 nitrite determinations (47 products x 6; 3 products x 5);
- 297 nitrate determinations (47 products x 6; 3 products x 5).

For each result obtained, the standard deviation was calculated and hence the standard error (giving the precision of the mean values) could be determined, allowing 95% confidence intervals.

The Reducing Capacity of all cadmium columns being operated throughout the survey was checked regularly and in general found to be higher than 90%. In the few instances when the reduction efficiency was somehow found to be lower than this limit, the glass column had to be emptied, regenerated with metallic cadmium powder and checked again. A very good indication of an acceptable
reducing capacity was a flow rate \( \leq 3 \text{ mL/min.} \)

9.3 **Precision, Accuracy & Possible Errors**

9.3.1 **Precision (Repeatability) of Method**

Precision is defined as the degree of agreement between replicate measurements of the same quantity, i.e. it gives the repeatability of a result (Christian, 1986). Good precision does not necessarily imply a high level of accuracy. However, the higher the degree of precision, the greater the chance of obtaining the true value.

In general, the more measurements taken, the more reliable will be the measure of precision. It follows that the precision of a measured value can be improved by increasing the number of observations.

9.3.1.1 **Estimated Standard Deviation**

There are various ways of indicating the precision of the analysis from a set of analytical results. One way of expressing precision is by calculating the Estimated Standard Deviation (SD) of a finite set of experimental data (generally \( N < 30 \)), as follows:
where: \( N \) = number of measurements;  
\( x_i \) = individual measurements;  
\( \bar{x} \) = mean of individual measurements = \( \frac{\sum x_i}{N} \);  
\( N - 1 \) = degrees of freedom.

Appendix A shows the mean values and standard deviations obtained for each of the 50 sets of nitrite and nitrate determinations carried out in this project.

The Coefficient of Variation (relative standard deviation) can also be determined by expressing the standard deviation as a percentage of the mean.

**9.3.1.2 Standard Error**

The Standard Error, called also the standard deviation of the mean, measures the precision of the mean of a series of \( N \) measurements. This is inversely proportional to the square root of \( N \) of the deviation of the individual values. Hence:

\[
\text{Standard Error} = \frac{SD}{\sqrt{N}}
\]

where: \( SD \) = estimated standard deviation;  
\( N \) = no. of measurements (i.e. no. of determinations per set).
The standard error for each set of determinations was calculated and results were included in Tables 19, 20 and 21.

### 9.3.1.3 Pooled Standard Deviation, $S_p$

The Pooled Standard Deviation is sometimes used to obtain an improved estimate of the precision of a method. Precision data of different sets of analyses can be pooled if the random error is assumed to be the same for each set.

The pooled standard deviation provides a more reliable estimate of the precision of a method than an estimate value obtained from a single set. It is defined as follows:

$$
S_p = \sqrt{\frac{\sum(x_{i1} - \bar{x}_1)^2 + (x_{i2} - \bar{x}_2)^2 + \ldots + \sum(x_{ik} - \bar{x}_k)^2}{N - k}}
$$

where:  
\(\bar{x}_1, \bar{x}_2, \bar{x}_k\) = means of each of k sets of analysis;  
\(x_{i1}, x_{i2}, x_{ik}\) = individual values in each set;  
\(N\) = total number of measurements *  
\(= N_1 + N_2 + \ldots + N_k\)  
\(N - k\) = degrees of freedom  
\(= (N_1 - 1) + (N_2 - 1) + \ldots + (N_k - 1)\)
* The number of samples in each set need not be equal. This equation combines the equations for the standard deviations of each set of data.

Data obtained from the 50 sets of nitrite determinations and from the equivalent number of nitrate analyses were computed in this equation and gave the following pooled standard deviations, as an indication of the overall precision of the method employed in the survey.

For nitrite determinations:

\[
N = \text{total number of determinations} = 297. \\
k = \text{number of degrees of freedom} = 50. \\
\therefore s_p = \sqrt{\frac{125.047}{247}} = 0.71 \text{ mg/kg}
\]

For nitrate determinations:

\[
N = 297. \\
k = 50. \\
\therefore s_p = \sqrt{\frac{8077.1631}{247}} = 5.72 \text{ mg/kg}
\]

Such results might give the impression that the method for analysis of nitrite had a higher precision than the one used for
nitrate determination (the latter having a higher value of $s_p$.
However, when such figures were expressed as a percentage of the
overall mean values for the survey (i.e. 7.5 mg/kg for nitrite and 62.5
mg/kg for the nitrate), the coefficient of variation of both methods
gave similar values. In fact:

For nitrite determinations:

Coefficient of Variation \[ \frac{s_p \text{(nitrite)}}{\bar{x} \text{(nitrite)}} \times 100\% \]

\[ \frac{0.71}{7.5} \times 100\% \]

\[ = 9.5\% \]

For nitrite determinations:

Coefficient of Variation \[ \frac{s_p \text{(nitrate)}}{\bar{x} \text{(nitrate)}} \times 100\% \]

\[ \frac{5.72}{62.5} \times 100\% \]

\[ = 9.2\% \]

9.3.1 Accuracy

Accuracy may be defined as the degree of agreement
between the measured value and an “accepted” true value (given that
the absolute true values are rarely known) (Christian, 1986).
One can arrive at a reasonable assumption on the accuracy of a method by comparing results with those of a known standard sample of similar composition, within the limitations of the knowledge of the "known" sample, and of measurements taken. In other words, accuracy depends on some measurement having a given limit of certainty in it.

In the case of meat products, the situation is quite complex because when nitrite is added to meat and analysed immediately after processing, only about 50% (sometimes even less) is detectable as residual nitrite by the usual analytical methods. This so-called residual or measurable nitrite level declines further during storage and distribution, as the product moves to the consumer for final preparation and consumption (Cassens, 1995). Such depletion is thought to be faster:

a) at abuse temperatures, e.g. room temp., than when product is refrigerated (Nordin, 1969);

b) in the presence of reductants (Fox & Nicholas, 1974).

Furthermore, the chemical forms of the residual nitrite detected by the analytical methods available are not fully known (although some believe it is in the form of bound nitric oxide); nor are the forms and reactivity of the added nitrite that is not detectable as
residual, including the portion of the added nitrite that could not be accounted for in some baseline studies.

In this project, a nitrite reference material was prepared by adding a known amount of sodium nitrite to a weighed sample of lean beef (as described in section 8.9.2) and assayed spectro-photometrically against a meat blank (refer to section 8.9.1) in the same way adopted for product samples. The procedure was repeated with a second fresh sample in a separate occasion.

The percentage recovery of residual nitrite was 49.1% in the first instance and 53.2% in the second occasion. On the other hand, the total percentage recovery of residual nitrite and nitrate was measured as 77.9%, indicating that residual nitrate is not depleted instantaneously to the same extent as its reactive nitrite counterpart.

However, as previously discussed, the overall accuracy of a method can only be determined by subjecting a number of samples of meat products to several inter-laboratory trials and analysing both the different sets of results.

9.3.3 Possible Errors Involved

The accuracy and precision of a method can be affected by 2 main types of errors: DETERMINATE and INDETERMINATE errors.
1) **DETERMINATE ERRORS**, are those errors that can probably be either avoided or corrected. They may be constant (e.g. use of uncalibrated weights) or variable (e.g. erroneous measurement of burette readings). Typical determinate errors (which may be additive or multiplicative) are:

- **instrumental errors**
  (e.g faulty equipment, uncalibrated weights/glassware, etc.);
- **operative errors**
  (e.g. human errors due to lack of experience and mathematical errors in calculations, biased measurements);
- **errors of the method**
  (e.g. errors due to side reactions, incomplete reactions, impurities in reagents, sparingly soluble precipitations, etc.)

The last type of determinate errors is considered as the most serious error of analysis (Christian, 1986), but can sometimes be corrected by running a reagent blank. It is standard practice to run a **blank determination** (analysing only added reagents) and then subtract results from those for the sample.

2) **INDETERMINATE ERRORS**, also called "accidental" or "random" errors, represent the experimental uncertainty occurring in any determination. Such errors, which follow a random distribution, are usually expressed by small differences in successive measurements made by the same analyst under virtually
identical conditions. Indeterminate errors cannot be predicted or estimated, and may only be quantified using mathematical laws of probability. They are usually considered to follow a normal distribution.

Indeterminate errors may arise from the limited ability of the analyst to control or make corrections to external conditions, or from pure statistics. It is practically impossible to eliminate all possible random errors in an experiment; however, the right approach would be to minimize them to a tolerable or insignificant level.

9.4 Survey Results

The results for the products analysed, as classified in the 3 categories described in section 9.2, are summarised in Tables 19, 20, 21. On the other hand, Tables 22, 23, 24 show the mean nitrite and nitrate concentrations per product type.

Detailed results for each product, showing means and standard deviations for both nitrite and nitrate are shown in Appendix A. Individual results of residual nitrate and nitrate content per sample, are listed in Appendix B.
Table 19

Mean Residual Nitrite & Nitrate Content (+ Standard Error of Mean) in:

**Category A: Canned Shelf-Stable Meat Products**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>expressed as NaNO₂</td>
<td></td>
<td>expressed as NaNO₃</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Chopped Ham with Pork A</td>
<td>Denmark</td>
<td>R 14</td>
<td>5.2</td>
<td>0.10</td>
<td>27.4</td>
<td>0.47</td>
</tr>
<tr>
<td>2.</td>
<td>Chopped Ham with Pork B</td>
<td>Denmark</td>
<td>R 20</td>
<td>1.8</td>
<td>0.13</td>
<td>38.1</td>
<td>2.39</td>
</tr>
<tr>
<td>3.</td>
<td>Chopped Ham with Pork C</td>
<td>Germany</td>
<td>R 13</td>
<td>5.2</td>
<td>0.20</td>
<td>30.4</td>
<td>1.55</td>
</tr>
<tr>
<td>4.</td>
<td>Corned Beef A</td>
<td>Argentina</td>
<td>R 18</td>
<td>5.2</td>
<td>0.05</td>
<td>29.0</td>
<td>0.87</td>
</tr>
<tr>
<td>5.</td>
<td>Corned Beef B</td>
<td>Brazil</td>
<td>R 01</td>
<td>9.5</td>
<td>0.35</td>
<td>29.7</td>
<td>1.93</td>
</tr>
<tr>
<td>6.</td>
<td>Corned Beef C</td>
<td>Brazil</td>
<td>R 11</td>
<td>8.5</td>
<td>0.47</td>
<td>35.7</td>
<td>2.21</td>
</tr>
<tr>
<td>7.</td>
<td>Corned Beef D</td>
<td>France</td>
<td>R 16</td>
<td>7.6</td>
<td>0.44</td>
<td>30.8</td>
<td>0.68</td>
</tr>
<tr>
<td>8.</td>
<td>Pork &amp; Stuffing Roll</td>
<td>England</td>
<td>R 07</td>
<td>6.0</td>
<td>0.33</td>
<td>39.7</td>
<td>1.34</td>
</tr>
<tr>
<td>9.</td>
<td>Pork Luncheon Meat A</td>
<td>China</td>
<td>R 02</td>
<td>5.6</td>
<td>0.11</td>
<td>19.8</td>
<td>0.86</td>
</tr>
<tr>
<td>10.</td>
<td>Pork Luncheon Meat B</td>
<td>China</td>
<td>R 08</td>
<td>6.8</td>
<td>0.10</td>
<td>23.8</td>
<td>1.31</td>
</tr>
<tr>
<td>11.</td>
<td>Pork Luncheon Meat C</td>
<td>Denmark</td>
<td>R 03</td>
<td>10.2</td>
<td>0.42</td>
<td>31.4</td>
<td>1.45</td>
</tr>
<tr>
<td>12.</td>
<td>Pork Luncheon Meat D</td>
<td>Denmark</td>
<td>R 06</td>
<td>7.1</td>
<td>0.33</td>
<td>28.9</td>
<td>2.00</td>
</tr>
<tr>
<td>13.</td>
<td>Pork Luncheon Meat E</td>
<td>England</td>
<td>R 09</td>
<td>8.5</td>
<td>0.27</td>
<td>33.3</td>
<td>1.27</td>
</tr>
<tr>
<td>14.</td>
<td>Pork Luncheon Meat F</td>
<td>Holland</td>
<td>R 15</td>
<td>6.6</td>
<td>0.46</td>
<td>36.6</td>
<td>2.17</td>
</tr>
<tr>
<td>15.</td>
<td>Reformed Ham</td>
<td>England</td>
<td>R 12</td>
<td>7.1</td>
<td>0.09</td>
<td>41.1</td>
<td>1.49</td>
</tr>
<tr>
<td>16.</td>
<td>Sausages (Chick. Hot Dogs) A</td>
<td>England</td>
<td>R 44</td>
<td>9.6</td>
<td>0.29</td>
<td>57.7</td>
<td>0.76</td>
</tr>
<tr>
<td>17.</td>
<td>Sausages (Cocktail) B</td>
<td>England</td>
<td>R 17</td>
<td>7.8</td>
<td>0.30</td>
<td>27.7</td>
<td>1.32</td>
</tr>
<tr>
<td>18.</td>
<td>Sausages (Frankfurters) C</td>
<td>Germany</td>
<td>R 46</td>
<td>8.1</td>
<td>0.28</td>
<td>45.0</td>
<td>1.11</td>
</tr>
<tr>
<td>19.</td>
<td>Sausages (Hot Dogs) D</td>
<td>Denmark</td>
<td>R 04</td>
<td>9.1</td>
<td>0.32</td>
<td>18.4</td>
<td>0.57</td>
</tr>
<tr>
<td>20.</td>
<td>Sausages (Hot Dogs) E</td>
<td>Holland</td>
<td>R 10</td>
<td>7.7</td>
<td>0.30</td>
<td>23.1</td>
<td>1.22</td>
</tr>
<tr>
<td>21.</td>
<td>Sausages (Hot Dogs) F</td>
<td>Holland</td>
<td>R 43</td>
<td>8.6</td>
<td>0.18</td>
<td>27.3</td>
<td>1.22</td>
</tr>
<tr>
<td>22.</td>
<td>Sausages (Hot Dogs) G</td>
<td>Holland</td>
<td>R 45</td>
<td>8.2</td>
<td>0.26</td>
<td>25.3</td>
<td>1.13</td>
</tr>
</tbody>
</table>
Table 20

Mean Residual Nitrite & Nitrate Content (+ Standard Error of Mean) in:

**Category B: Refrigerated Meat Products**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>Gammon (Loose)</td>
<td>Malta</td>
<td>R47</td>
<td>11.0</td>
<td>0.26</td>
<td>130.6</td>
<td>± 2.99</td>
</tr>
<tr>
<td>24</td>
<td>Gammon Joint</td>
<td>Malta</td>
<td>R50</td>
<td>7.7</td>
<td>0.39</td>
<td>19.2</td>
<td>0.69</td>
</tr>
<tr>
<td>25</td>
<td>Ham</td>
<td>Malta</td>
<td>R19</td>
<td>6.4</td>
<td>0.45</td>
<td>33.1</td>
<td>2.45</td>
</tr>
<tr>
<td>26</td>
<td>Mortadella (Sausage Type) A</td>
<td>Malta</td>
<td>R05</td>
<td>5.8</td>
<td>0.17</td>
<td>162.7</td>
<td>6.93</td>
</tr>
<tr>
<td>27</td>
<td>Mortadella B</td>
<td>Malta</td>
<td>R22</td>
<td>6.1</td>
<td>0.35</td>
<td>134.7</td>
<td>5.81</td>
</tr>
<tr>
<td>28</td>
<td>Salami (Loose) A</td>
<td>Denmark</td>
<td>R48</td>
<td>1.6</td>
<td>0.08</td>
<td>22.2</td>
<td>1.34</td>
</tr>
<tr>
<td>27</td>
<td>Salami (Smoked) B</td>
<td>Denmark</td>
<td>R49</td>
<td>1.8</td>
<td>0.06</td>
<td>51.4</td>
<td>0.06</td>
</tr>
<tr>
<td>30</td>
<td>Sausages (Chick &amp; Turk Franks)</td>
<td>Italy</td>
<td>R42</td>
<td>7.3</td>
<td>0.06</td>
<td>86.4</td>
<td>2.07</td>
</tr>
<tr>
<td>31</td>
<td>Sausages (Maltese Type)</td>
<td>Malta</td>
<td>R21</td>
<td>0.1</td>
<td>0.03</td>
<td>13.6</td>
<td>1.18</td>
</tr>
</tbody>
</table>

Determination of Residual Levels of Nitrites & Nitrates in Meat Products
Table 21
Mean Residual Nitrite & Nitrate Content (+ Standard Error of Mean) in:

**Category C: Frozen Meat Products**

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>expressed as NaN02</td>
<td>expressed as NaN02</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32.</td>
<td>Bacon (Back) A</td>
<td>Malta</td>
<td>R 23</td>
<td>55.9</td>
<td>0.95</td>
<td>49.7</td>
<td>1.50</td>
</tr>
<tr>
<td>33.</td>
<td>Bacon (Collar) B</td>
<td>Malta</td>
<td>R 24</td>
<td>3.7</td>
<td>0.16</td>
<td>487.4</td>
<td>6.42</td>
</tr>
<tr>
<td>34.</td>
<td>Bacon (Streaky) C</td>
<td>Malta</td>
<td>R 29</td>
<td>19.0</td>
<td>0.65</td>
<td>76.6</td>
<td>1.42</td>
</tr>
<tr>
<td>35.</td>
<td>Beef Burgers A</td>
<td>Malta</td>
<td>R 27</td>
<td>0.3</td>
<td>0.03</td>
<td>35.9</td>
<td>2.52</td>
</tr>
<tr>
<td>36.</td>
<td>Beef Burgers B</td>
<td>Malta</td>
<td>R 28</td>
<td>0.2</td>
<td>0.03</td>
<td>22.6</td>
<td>1.35</td>
</tr>
<tr>
<td>37.</td>
<td>Sausages (BarBQ/Grill) A</td>
<td>Malta</td>
<td>R 41</td>
<td>0.2</td>
<td>0.02</td>
<td>35.2</td>
<td>0.54</td>
</tr>
<tr>
<td>38.</td>
<td>Sausages (Beef) B</td>
<td>Malta</td>
<td>R 30</td>
<td>0.0</td>
<td>0.00</td>
<td>74.6</td>
<td>2.25</td>
</tr>
<tr>
<td>39.</td>
<td>Sausages (Beef) C</td>
<td>Malta</td>
<td>R 34</td>
<td>0.0</td>
<td>0.00</td>
<td>55.8</td>
<td>2.80</td>
</tr>
<tr>
<td>40.</td>
<td>Sausages (Chick/Turk Franks) D</td>
<td>Malta</td>
<td>R 39</td>
<td>5.5</td>
<td>0.19</td>
<td>157.8</td>
<td>2.29</td>
</tr>
<tr>
<td>41.</td>
<td>Sausages (Frankfurters) E</td>
<td>Malta</td>
<td>R 26</td>
<td>2.4</td>
<td>0.08</td>
<td>27.6</td>
<td>1.35</td>
</tr>
<tr>
<td>42.</td>
<td>Sausages (Frankfurters) F</td>
<td>Malta</td>
<td>R 31</td>
<td>38.7</td>
<td>0.40</td>
<td>164.3</td>
<td>2.98</td>
</tr>
<tr>
<td>43.</td>
<td>Sausages (Frankfurters) G</td>
<td>Malta</td>
<td>R 32</td>
<td>8.6</td>
<td>0.32</td>
<td>212.4</td>
<td>3.06</td>
</tr>
<tr>
<td>44.</td>
<td>Sausages (Frankfurters) H</td>
<td>Malta</td>
<td>R 40</td>
<td>8.3</td>
<td>0.07</td>
<td>59.8</td>
<td>0.89</td>
</tr>
<tr>
<td>45.</td>
<td>Sausages (Pork) I</td>
<td>Malta</td>
<td>R 25</td>
<td>0.0</td>
<td>0.00</td>
<td>63.6</td>
<td>4.03</td>
</tr>
<tr>
<td>46.</td>
<td>Sausages (Pork) J</td>
<td>Malta</td>
<td>R 33</td>
<td>0.0</td>
<td>0.00</td>
<td>57.7</td>
<td>2.66</td>
</tr>
<tr>
<td>47.</td>
<td>Sausages (Others) K</td>
<td>Malta</td>
<td>R 35</td>
<td>7.9</td>
<td>0.26</td>
<td>58.7</td>
<td>0.85</td>
</tr>
<tr>
<td>48.</td>
<td>Sausages (Others) L</td>
<td>Malta</td>
<td>R 36</td>
<td>6.1</td>
<td>0.22</td>
<td>47.6</td>
<td>1.75</td>
</tr>
<tr>
<td>49.</td>
<td>Sausages (Others) M</td>
<td>Malta</td>
<td>R 37</td>
<td>10.2</td>
<td>0.15</td>
<td>56.2</td>
<td>0.31</td>
</tr>
<tr>
<td>50.</td>
<td>Sausages (Others) N</td>
<td>Malta</td>
<td>R 38</td>
<td>0.2</td>
<td>0.10</td>
<td>28.2</td>
<td>0.76</td>
</tr>
</tbody>
</table>
Table 22

Mean Nitrite and Nitrate Concentrations per Product Type:

**Category A - Canned Shelf-Stable Meat Products**

(Product Nos: 1-22)

<table>
<thead>
<tr>
<th>Type</th>
<th>Product</th>
<th>Frequency</th>
<th>Mean Nitrite (ppm)</th>
<th>Mean Nitrate (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Chopped Ham with Pork</td>
<td>3</td>
<td>4.1</td>
<td>32.0</td>
</tr>
<tr>
<td>A2</td>
<td>Corned Beef</td>
<td>4</td>
<td>7.7</td>
<td>31.3</td>
</tr>
<tr>
<td>A3</td>
<td>Pork &amp; Stuffing Roll</td>
<td>1</td>
<td>6.0</td>
<td>39.7</td>
</tr>
<tr>
<td>A4</td>
<td>Pork Luncheon Meat</td>
<td>6</td>
<td>7.5</td>
<td>29.0</td>
</tr>
<tr>
<td>A5</td>
<td>Reformed Ham</td>
<td>1</td>
<td>7.1</td>
<td>41.1</td>
</tr>
<tr>
<td>A6</td>
<td>Sausages (include. Hot Dogs/Franks.)</td>
<td>7</td>
<td>8.4</td>
<td>32.1</td>
</tr>
</tbody>
</table>

Table 23

Mean Nitrite and Nitrate Concentrations per Product Type:

**Category B: Refrigerated Meat Products**

(Product Nos: 23-31)

<table>
<thead>
<tr>
<th>Type</th>
<th>Product</th>
<th>Frequency</th>
<th>Mean Nitrite (ppm)</th>
<th>Mean Nitrate (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>Gammon</td>
<td>1</td>
<td>11.0</td>
<td>130.6</td>
</tr>
<tr>
<td>B2</td>
<td>Gammon Joint</td>
<td>1</td>
<td>7.7</td>
<td>19.2</td>
</tr>
<tr>
<td>B3</td>
<td>Lham</td>
<td>1</td>
<td>6.4</td>
<td>33.1</td>
</tr>
<tr>
<td>B4</td>
<td>Mortadella</td>
<td>2</td>
<td>6.0</td>
<td>148.7</td>
</tr>
<tr>
<td>B5</td>
<td>Salami</td>
<td>2</td>
<td>1.1</td>
<td>36.8</td>
</tr>
<tr>
<td>B6</td>
<td>Sausages (Frankfurters)</td>
<td>1</td>
<td>7.2</td>
<td>88.4</td>
</tr>
<tr>
<td>B7</td>
<td>Sausages (Maltese Type)</td>
<td>1</td>
<td>0.1</td>
<td>13.6</td>
</tr>
</tbody>
</table>

Table 24

Mean Nitrite and Nitrate Concentrations per Product Type:

**Category A: Frozen Meat Products**

(Product Nos: 32-50)

<table>
<thead>
<tr>
<th>Type</th>
<th>Product</th>
<th>Frequency</th>
<th>Mean Nitrite (ppm)</th>
<th>Mean Nitrate (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>Bacon (Collar, Back, Streaky)</td>
<td>3</td>
<td>26.2</td>
<td>204.6</td>
</tr>
<tr>
<td>C2</td>
<td>Sausages (inc. Pork, Beef, Frankfurters)</td>
<td>14</td>
<td>6.3</td>
<td>78.5</td>
</tr>
<tr>
<td>C3</td>
<td>Beef Burgers</td>
<td>2</td>
<td>0.3</td>
<td>29.3</td>
</tr>
</tbody>
</table>
9.5 Discussion

The majority of products contained concentrations of nitrite and nitrate far below the maximum permitted levels laid down in both the 1994 and 1998 Regulations (Govt. of Malta, 1994b; Govt. of Malta, 1998b).

9.5.1 Category A: Canned Products

Results in Table 19 show that all canned meat products had low levels of nitrite, the lowest being 1.8 mg/kg (in chopped ham with pork B) and the highest value being 10.2 mg/kg (in pork luncheon meat C), but still well below the maximum permitted level of 100 mg/kg of nitrite for canned products in the 1998 regulations (Govt. of Malta, 1998b). According to results in Table 22, the mean residual sodium nitrite content per canned product type varied from 4.0 to 8.4 mg/kg.

A similar trend seems to apply for residual nitrate found in the same canned products. In fact, Table 19 shows that sodium nitrate levels in such products ranged from 18.4 to 57.7 mg/kg, which is well below the maximum of 250 mg/kg sodium nitrate allowed for
canned products in the new regulations. Table 22 further specifies that the mean nitrite level per product type varied only between 29.0 and 41.1 mg/kg.

9.5.2 Category B: Refrigerated Products

Results of nitrite concentrations in refrigerated (fresh) products listed in Tables 20 and 23, show more or less the same trend of residual levels found in canned products, with the level ranging from 0.1 mg/kg (found in the practically "uncured" Maltese-type sausages) to 11.0 mg/kg nitrite (found in gammon).

In the case of sodium nitrate content, the levels in refrigerated products were higher, as compared with canned products, with three local products exceeding the 100 mg/kg mark. High residual nitrate content were in fact recorded in gammon (130.6 mg/kg) and two types of mortadella (162.7 and 134.7 mg/kg nitrite). These levels were however still below the maximum level (250 ppm) stipulated in the 1998 Regulations and the combined maximum amount of nitrite and nitrate in cured meats (500 ppm) cited in the former (1994) Regulations.

9.5.3 Category C: Frozen Products

The most interesting results were certainly those
obtained from the analysis of frozen meat products (Tables 21 and 24) with several products containing virtually no residual nitrite, while others literally (and relatively) abounding in either or both preservatives.

Residual sodium nitrite was in fact found to exceed 30 mg/kg in two products (bacon A and sausages F), the highest value (55.9 mg/kg in the case of bacon A) still below the maximum permitted limits laid down by both the 1994 and 1998 Regulations. On the other hand, 4 types of sausages (B, C, I, J) were found to possess no nitrite content at all, while only trace amounts (≤ 0.3 mg/kg) of nitrite could be detected in 4 other products (beef burgers A & B; sausages A & N). This means that unless other suitable antibotulinal agents were present, these 8 products were at risk for infection by *Clostridium botulinum* (and possibly by other pathogenic/spoilage organisms), in spite of the low storage temperature.

There were 4 products with relatively high nitrate level, all exceeding 150 mg/kg. While three of these products (i.e. sausages D, F and G) appeared to be within limits imposed by law, the fourth product (i.e. collar bacon B) had a mean nitrite content of 487.4 mg/kg which is practically twice the maximum value allowed by the
Permitted Food Additives Regulations, 1998 (Govt. of Malta, 1998b) (i.e. 250 mg/kg nitrate).

This result could however be contested on the basis of the processing and/or "best before" date since one might argue that legal provisions of the 1998 Regulations were not applicable to a product processed prior to 1 January 1999 (the date when the 1998 Regulations came in force). If that is the case, the previous regulations, i.e., the Preservatives in Food Regulations, 1994 would apply and the result obtained would not have reached the maximum total allowance of sodium nitrate and sodium nitrate (for bacon, whether cooked or uncooked) by a mere 8.9 mg/kg. In fact, the combined maximum limit of both preservatives was fixed at 500 mg/kg while the product was found to have a total concentration of 491.1 mg/kg of residual nitrite and nitrate.

The latter result, which is being referred to the local Public Health authorities, is definitely a case for further consideration and would probably have to be treated as a borderline case, pending confirmatory results on the same product (possibly from the same batch) from other laboratories.
9.6 Interpretation

It must be pointed out that the standard method used in this survey was found to have a total (nitrite and nitrate) recovery of 77.9%, comparing well but still below the value obtained by a similar method proposed by Binstok et al. (1996), based on the modified Norwitz-Kelliher extraction technique (Norwitz & Kelliher, 1986), which was found to have a total recovery rate of 83.0%, when checked with the same in-house reference material. Cassens et al. (1974) argue that nitrite recovery is expected to be particularly low, even if a meat slurry having a fixed amount of nitrite is analysed immediately upon addition of the salt. The same authors sustain that nitrite would disappear even more quickly with heating and the amount would decline upon subsequent storage.

It is also reported that only between 50-75% of the originally added nitrite can be measured immediately after formulation, by analytical procedures (Perez-Rodriguez et al., 1996). Subsequent thermal processing is expected to result in an additional nitrite loss of 20 to 80%, with the loss continuing during storage (Cassens et al., 1977). This is precisely why the term “residual nitrite” is often used to interpret results from surveys similar to the one carried out in this project. Residual nitrite refers to the amount of
nitrite current analytical methodology can detect in a cured meat product.

Although reference to residual nitrite and nitrate, or a measure of free nitrite and nitrate, in a cured meat offers something tangible to consider when the balance between food additives and human health is weighed, the accuracy of the analytical procedures in determining such residual levels remains in question.

Having said that, results originating from this survey reveal, if any, that the concentrations of nitrite and nitrate (at least, the “measurable” residual contents) were generally below 50% of their current maximum permitted level. Furthermore, it could be deduced that:

i) 88% of products analysed contained less than 10% (i.e. < 10 ppm) of the maximum allowed limit of nitrite;

ii) 64% of products contained less than 25% (i.e. < 50 ppm) of the current legal limit of nitrate, with higher levels found mainly in the frozen products.

Another debatable point is the low level or complete absence of nitrite in some of the meat products analysed. Such levels may be below a concentration that, unaided, would normally be
expected to effectively control microbiological contamination. However, some of the pre-packaged samples were found to include other additives such as sodium ascorbate (used as an adjunct to nitrite, to prevent formation of nitrosamines) as an additional antioxidant, amongst the ingredients declared on the label. The presence of such antioxidant, as well as salt (sodium chloride) is believed to reduce risks associated with microbiological safety of the products concerned.

In general terms, the data indicated that:

(A) concentrations of NITRITE ranged
   ♦ from 1.8 to 10.2 mg/kg in canned meat products;
   ♦ from < 0.3 to 11.0 mg/kg in refrigerated products;
   ♦ from < 0.3 to 55.9 mg/kg in frozen products (inc. bacon);

(B) levels of NITRATE ranged as follows:
   ♦ from 18.4 to 57.7 mg/kg in canned meat products;
   ♦ from 13.6 to 162.7 mg/kg in refrigerated products;
   ♦ from 22.6 to 487.4 mg/kg in frozen products (inc. bacon).

The following were the Overall Mean Nitrite and Mean Nitrate concentrations in the 3 categories of cured products under investigation.
Table 25

Mean Levels of Nitrite & Nitrate in Different Categories of Cured Meat Products

<table>
<thead>
<tr>
<th>Category</th>
<th>Mean NaNO₂ (mg/kg)</th>
<th>Mean NaNO₃ (mg/kg)</th>
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<tr>
<td>A. Canned Shelf-Stable Products</td>
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<td>5.3</td>
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<tr>
<td>C. Frozen Products</td>
<td>8.8</td>
<td>93.2</td>
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</table>

On the basis of such findings, the overall average concentration was found to be 7.5 mg/kg, comparing well with the overall mean of 6.7 mg/kg nitrite obtained in the 1996 Survey on Cured Meat Products carried out by the local Public Health Laboratories (Public Health Labs., Malta, 1996). The overall nitrate value was established at 62.5 mg/kg. These mean values for nitrite and nitrate were well within the statutory limits and compare also well with values obtained in a similar Survey of Nitrite and Nitrate in Cured Meat Products in the British market, conducted by the Joint Food Safety & Standards Group within the UK Ministry of Agriculture, Fisheries & Food (1998).
10.1 Significance of Baseline Studies

Health authorities are in continuous need of scientifically reliable data upon which they can base their policies, decisions and actions. Baseline studies are therefore of paramount importance because they serve to identify and address problem cases related with particular aspects of health including safety and quality of food.

Results from such studies usually deliver valuable conclusions and/or recommendations, thereby providing any interested agencies/parties with useful information which may be exploited, for instance, in drawing up national or international strategies such as a "Food Control Strategy" to monitor those problems arising from the ever-growing movement of foods from one area to another and their eventual consumption.
10.2 **Area of Interest**

The area of interest chosen for this particular project was the determination of residual levels of nitrite and nitrate in locally processed and imported meat products. The following are just three of the main reasons behind such choice:

I. the widespread concern about the safety of nitrite-cured meat products due to the implication of nitrites and nitrates in the formation of nitroso compounds, some of which are specific and potent carcinogens;

II. the availability of standard methods of analysis that require equipment that is readily available, not sophisticated and reasonably reliable;

III. the possible lack of technical expertise of some local producers of meat products in the application of the curing technique, resulting in potential threats to the health of the local consumer.

10.3 **The “Nitrite Problem”**

10.3.1 **Functions of Nitrites and Nitrates in Meat Products**

In spite of the long and still outstanding controversy over the use of nitrite (and to a lesser extent nitrate) as a curing agent
during the manufacture of meat products, they are still added to a wide variety of cured meats. The main curing agent is sodium nitrite but nitrate serves as a reservoir for the production of nitrite ‘in situ’, through bacterial reduction of nitrate, and is primarily used in the curing of foods such as fermented sausages and dry-cured meats that require long production times.

Nitrite preserves meat by exerting a number of functions via its:

- antimicrobial role (by inhibiting pathogens, mainly *Clostridium botulinum* and several spoilage organisms);
- antioxidant effects (inhibiting even lipid oxidation); and
- sensory properties (by forming characteristic desirable colour and flavour, typical of cured meat).

10.3.2 Formation & Carcinogenicity of Nitrosamines

Nitrite is however a reactive chemical to be used with caution (Cassens, 1995). It was found to interact with various amino compounds to produce potentially carcinogenic N-nitroso compounds (e.g. nitrosamines and nitrosamides). This has caused considerable concern over possible adverse effects on public health and has resulted in increased pressure to reduce exposure of humans to these agents from all sources, including nitrite in cured meat.
Chapter 10

CONCLUSION

Local legislation (in accordance with EU and other regulations) currently allows up to 50 mg/kg or 100 mg/kg of residual nitrite in cured meat products (depending on the type of curing process) and a maximum of 175 mg/kg in cured bacon. On the other hand, the maximum residual level of nitrate in all cured and canned meat products presently stands at 250 mg/kg (expressed as sodium nitrate) (Govt. of Malta, 1998b).

10.3.3 Sources of Nitrite & Nitrate

A study commissioned by the US Assembly of Life Sciences (1982) on the average US citizen, reported that dietary nitrite originated mainly from cured meats (c. 39%) and baked goods and cereals (c. 34%) while ingested nitrates came primarily from vegetables (c. 87%). However, nitrate may be converted to nitrite in the human body and so the real sources of nitrite are vegetables (~72%), with less than an overall 10% deriving from cured meats. All this has to be taken into consideration before determining the ultimate possible limit of residual nitrite that can still safeguard the antibotulinal properties of the curing salt.

Although there is no doubt about the contribution of nitrite to the development of colour and flavour in cured meats, the concentration required to impart such characteristics is still not known (although it is known that sodium nitrite at 40-50 mg/kg is
sufficient to produce adequately stable cured-meat colour in most products).

10.4 **Analysing Meat Products for Residual Nitrite & Nitrate: Techniques & Problems**

Nitrite added to meat for the purpose of curing, can only be partially recovered in the finished product as residual nitrite (defined by Cassens (1974) as the amount of nitrite current analytical methodology can detect in a cured meat product). The remaining part combines with pigments or undergoes other reactions with several meat components (Perez-Rodriguez, 1996).

Measurement of the levels of residual or free nitrite in cured meat gives a point of reference in the never-ending debate on the possible human health risks in the use of such food additive.

Meat products can be analysed for nitrite and nitrate by a number of methods:

(1) **NITRITE** is usually converted to nitrous acid and reacted with a 1° aromatic amine to form a diazonium salt. This is then coupled with an aromatic compound having an influential amino or hydroxyl substituent to form an azo colour which can be assayed spectrophotometrically.
(2) NITRATE levels are usually determined by techniques involving one of the following reactions:

(a) reduction of nitrate to nitrite;
(b) oxidation of an organic compound by the nitrate ion;
(c) nitration of a phenolic compound;
(d) reduction of nitrate to ammonia.

Standard methods are usually based on reduction of nitrate to nitrite using a number of reagents such as copper, zinc, hydrazine sulphate and cadmium. The cadmium technique is the most popular and one of the most reliable methods considering that most of the other reducing agents give either incomplete reduction or further reduction of the nitrate ion to ammonia (resulting in variable recoveries).

Various other methods for the determination of nitrite and nitrate content in meat have been developed. One rapid method for the simultaneous determination of nitrite and nitrate ions in food, which seems to be gaining popularity, involves the use of HPLC, to separate the ions, coupled by detection by UV absorption.

Although most of these methods, especially those for the determination of nitrite, seem to be simple, results obtained from such methods show a greater variability than would be reasonably expected. Frouin (1977) affirms that the standard methods of
measuring nitrates and nitrites do not give accurate results and hence, analysts should be cautious in interpreting data.

In the case of nitrite, the problem lies mainly in its relatively low recovery from meat products due to a number of factors mainly related to its low stability and high reactivity with other components of meat. In fact, it has been shown that when nitrite is added to a meat slurry, immediate analysis would only recover up to 50% of the nitrite. Furthermore, it seems that nitrite disappears more quickly with heating and declines during storage. It has also been indicated that only 50-75% of the originally added nitrite (determined immediately after formulation) can be measured by analytical procedures available. Loss of nitrite is expected to continue during subsequent thermal processing (20-80%) and storage of product. All this explains why a typical cured product having about 150 ppm of added nitrite may end up with a residual level between 10-50 ppm.

10.5 Outcome from Survey Data

Results obtained in the survey conducted on 50 local and imported meat products indicate that the residual nitrite level of locally consumed products ranges from 1-11 mg/kg (well within the regulatory limit of 100 mg/kg for cured/canned meat products), excluding:
(i) Maltese-type sausages and beef burgers (survey mean value ≤ 0.3 mg/kg of nitrite), which are usually processed with sulphur dioxide rather than nitrite as an antimicrobial agent;

(ii) Bacon, which traditionally contains a higher amount of curing salts (survey mean value of 26.2 mg/kg; individual values being 3.7, 19.0 and 55.9 mg/kg). Cured bacon, in fact has a higher permitted maximum level of 175 mg/kg (with respect to other cured meat products).

On the other hand, 64% of products were found to have nitrate level inferior to 50 mg/kg, 22% contained 50-100 mg/kg, 10% fell in the range 100-200 mg/kg, while only 4% (2 cases) had more than 200 mg/kg of nitrate. The highest recorded level of nitrate was that found in locally cured collar bacon, which was found to have 487.4 mg/kg (± 6.42 mg/kg). This level exceeds the maximum permitted by current legislation (1998 Regulations) which stands at 250 mg/kg - almost by a factor of 2. However, the legal implications of this case are debatable considering that the survey was carried out in early 1999, only a few weeks/months from the effective date (1.1.99) when the new regulations became legally binding. Former regulations, applicable till 31.12.98, postulated a combined nitrate/nitrite maximum level of 500 mg/kg (with not more than 200 mg/kg of sodium nitrite) for bacon and ham products.
This case of high levels of nitrate definitely needs further treatment and is to be referred to the Health authorities for confirmation of results and possible investigations.

Investigations carried out throughout survey confirm other important observations reported in the literature, namely that:

- during reduction of nitrate to nitrite using a cadmium column, the rate of flow has to be rigorously controlled;
- the reducing capacity (and hence efficiency) of this column has to be regularly checked and maintained beyond 90%;
- the recovery of the ingoing (originally added) amount of nitrate and nitrite varies significantly and is very difficult to reproduce because of side reactions with components of meat, which use up the same nitrate and nitrite;
- one has to focus on the limiting levels of "residual" nitrate and nitrite in meat products after taking into consideration the above observations in order to protect the consumer.

10.6 Reliability of Results

Reliability of data is usually achieved through validation of the method adopted, by establishing both the precision and accuracy of the method.
Precision of results could be assessed by measuring the standard deviation and the standard error for every set of determinations. The overall precision of the method was then estimated by calculating the pooled standard deviations (for both nitrite and nitrate) from the results obtained in the 297 nitrite and 297 nitrate assays carried out in this project. The values obtained gave similar coefficient of variations, 9.5% in the case of nitrite and 9.2% in the case of nitrate.

The measurement of accuracy is more complex since it requires the comparison of results from standard reference material. The problem lies mainly in the high reactivity of nitrite in the meat matrix, bringing down immediately the level of the "measurable" or "residual" amount of preservative in a freshly prepared sample to about 50% of the added amount. Such recovery rate was in fact achieved and measured on two different occasions (49.1% and 53.2%) applying the same method of determination of nitrite on 2 separate samples of beef, comparing results in each case against those of a meat blank (taken to be the control mixture).

Nitrate results were found to be more reliable, considering a total recovery rate of nitrate and nitrite of 77.9% when applying the nitrite and nitrate assays consecutively on a freshly
prepared sample of reference beef (containing known amounts of sodium nitrite and sodium nitrate).

The true accuracy of the method can only be tested by cross-checking results (of the same product samples and reference materials) with those obtained by other laboratories, using the same procedures and equipment.

10.7 Further Studies on the Fate of Nitrite and Nitrate

In spite of the valuable work carried out by the US Assembly of Life Sciences (1981, 1982), Frouin (1977), Cassens et al. (1977), Cassens (1995, 1997) and others, one main issue that needs to be addressed in further studies of this nature is the real fate of added nitrate and nitrite. It has already been said that only from 50 to 70% of added nitrite is recovered, even when a standard preparation of a meat product is analysed immediately after formulation. The question remains: what happens to the rest? Is it the fault of the extraction method which fails to extract all the nitrite (and nitrate) or is it solely attributed to the maze of reactions involving components of the products, as reported in the literature?

One way of confirming this and establish the detailed fate
of added nitrite and nitrate would be the use of radioactive nitrogen in the curing mixture, separation of the product into the various components (e.g. protein and lipid fractions) and determination of radioactive nitrogen in the separate fractions. It appears that such work, complementing the nitrogen-15 tracer studies of nitrite drawn up by Sebranek et al. (1973), is the next step required towards the complete unfolding of the mystery of the “disappearing nitrite and nitrate” in meat products.

10.8 Alternatives to the Use of Nitrite & Nitrate

There are two general categories of alternatives to the conventional use of nitrite, namely:

I. agents or treatments that serve as partial or complete replacements for nitrite;

II. agents that block the formation of nitrosamines in products containing conventional amounts of nitrite.

A great deal of work has been done to assess the ability of alternatives in the first category to replace completely or partially the nitrite content in cured meat. Most of the efforts concentrated only on replacements for the antibotulinal role of nitrite. However, it has
now been realised that in order to achieve all the actions exerted by nitrite, combinations of compounds are required. Such combinations may, for example, include small amounts of nitrite (e.g. 40-50 mg/kg), sufficient to develop the cured meat pigment.

According to the Committee on Nitrite & Alternative Curing Agents in Food (US Assembly of Life Sciences, 1982), considering the full role of nitrite in meat, the most promising alternatives to the use of this preservative seem to be:

(i) a combination of ascorbate, α-tocopherol and nitrite;
(ii) irradiation (with or without nitrite);
(iii) lactic-acid-producing organisms (with or without nitrite);
(iv) potassium sorbate with low concentrations of nitrite;
(v) sodium hypophosphite (with or without nitrite), and
(vi) several fumarate esters.

All these alternatives, with the exception of ascorbate and α-tocopherol, serve mainly as partial or complete replacements for the antimicrobial role of nitrite. On the other hand, ascorbate and α-tocopherol function by inhibiting nitrosamine formation.

The main reasons why only a limited number of possible compounds can substitute for the antibotulinal role of nitrite in cured meat are:
(a) toxicological considerations;
(b) cost effectiveness;
(c) practicability in application;
(d) concentrations necessary for inhibition;
(e) effects on various quality characteristics of the product.

It seems that nitrates are already being gradually phased out as meat preservatives, its role being effectively taken by other salts such as sodium chloride. Yet reduced amounts (with respect to former curing practices) are still included sometimes to serve as potential store for replenishment (by microbial reduction) of the very reactive and hence unstable nitrite ion.

In spite of the seemingly unending debate and dilemmas on the use of nitrite and nitrate in cured meat products, nitrite is still considered as an important requisite to secure the safety of the products concerned. However, it is equally important to keep on focussing on developing suitable alternatives to substitute for one or more functions of nitrite, thereby reducing levels of the curing salts to the lowest possible to eliminate any remote risk from the inevitable formation of the problematic N-nitroso compounds.
References


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## Survey Reports

Mean Levels of Sodium Nitrite & Sodium Nitrate, with Standard Deviations
(both nitrite & nitrate, expressed in mg/kg of the sodium salt)

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<tr>
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<th>( \text{NO}_3^- )</th>
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## Appendix B

### Survey into the Levels of Nitrite & Nitrate in Meat Products - 1999

(both nitrite & nitrate, expressed in mg/kg of the sodium salt)

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Determination of Nitrite & Nitrate in Meat Products

Data

Report Number: _____ Date: _____________ Time: _____________
Product Type: _______________ Brand Name: _______________
Country of Origin: _____________ Net Weight: _______________
Other Details: _______________________________________

Calibration Curve

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<th>Conc. of Nitrite (µg/L)</th>
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Calculation of N (NITRITE)

Nitrite Content, N, expressed in mg/kg of NaNO₂ is calculated using formula:

\[
N = 200 \frac{m_1}{m_0 V_f}
\]

where 

- \( m_0 \) = mass in g of test portion (sample)
- \( m_1 \) = value given by Calibration Curve for mass of NaNO₂ in µg, corresponding to absorbance of the test solution (filtrate)
- \( V_f \) = volume in ml of the aliquot portion of filtrate taken for test
Appendix C (SPECIMEN REPORT SHEET)

Calculation of S (NITRATE)

Nitrate Content, S, expressed in mg/kg of NaNO₃ is calculated using formula:

\[
S = 1.203 \left( \frac{1000m_2}{m_0 V_c} - N \right)
\]

where

- \( m_0 \) = mass in g of test portion (sample)
- \( m_2 \) = value given by Calibration Curve for mass of NaNO₂ in μg, corresponding to absorbance of the test solution (filtrate)
- \( N \) = nitrite content of sample (in mg/kg NaNO₂)
- \( V_c \) = volume in ml of the aliquot portion of effluent (from Reduction Column) taken for the colorimetric measurement.

Conclusions

____________________________________________________________________________________

____________________________________________________________________________________

____________________________________________________________________________________

Comments

____________________________________________________________________________________

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____________________________________________________________________________________

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____________________________________________________________________________________

____________________________________________________________________________________
## Determination of Nitrite and Nitrate in Meat Products

### Results

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<th>Sample</th>
<th>Mass $m_0$ (g)</th>
<th>Absorb. @ 538 nm</th>
<th>Mass of Nitrite $m_1$ (µg)</th>
<th>Volume $V_f$ (ml)</th>
<th>Nitrite Content $N$ (mg/kg)</th>
<th>Absorb. @ 538 nm</th>
<th>Mass of Nitrite $m_2$ (µg)</th>
<th>Volume $V_c$ (ml)</th>
<th>Nitrate Content $S$ (mg/kg)</th>
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Product: ___________  Report No: _______  Date: _______
Appendix D

Foodstuffs Regulations Currently in Force

(27 May, 1999)

Food Hygiene Regulations, 1969 (L.N. 73/69)
Importation & Use of Brominated Vegetable Oils (Prohibition) Regulations, 1972 (L.N. 9/72)
Food Hygiene (Amendment) Regulations, 1973 (L.N. 77/73)
Food Hygiene (Amendment) Regulations, 1974 (L.N. 135/74)
Public Markets Regulations, 1975 (L.N. 141/75)
Dairy Farms Regulations, 1976 (L.N. 28/76)
Lead in Cooking Utensils Regulations, 1976 (L.N. 91/76)
Inspection of Milk Animals Regulations, 1976 (L.N. 92/76)
Fruits, Legumes and Vegetables Regulations, 1977 (L.N. 8/77)
Diethyl Pyrocarbonate (Control) Regulations, 1977 (L.N. 9/77)
Meat Regulations, 1977 (L.N. 15/77)
Sale of Fish Regulations, 1977 (L.N. 19/77)
Public Markets (Amendment) Regulations, 1977 (L.N. 20/77)
Food Hygiene (Amendment) Regulations, 1977 (L.N. 25/77)
Beer (Sale) Regulations, 1977 (L.N. 26/77)
Meat Importation Regulations, 1977 (L.N. 28/77)
Edible Fats and Oils Regulations, 1977 (L.N. 42/77)
Flour, Bread and Paste Regulations, 1977 (L.N. 92/77)
Canned Food Regulations, 1977 (L.N. 142/77)
Sale of Pasteurised Milk Regulations, 1977 (L.N. 148/77)
Sausages, Salted Meats and Prepared Meat Regs, 1977 (L.N. 156/77)
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Soft Drinks Regulations, 1978 (L.N. 13/78)
Flour, Bread and Paste (Amendment) Regulations, 1978 (L.N. 32/78)
Soft Drinks (Amendment) Regulations, 1978 (L.N. 33/78)
Edible Fats and Oils (Amendment) Regulations, 1978 (L.N. 61/78)
Corned Beef Regulations, 1981 (L.N. 100/81)
Luncheon Meat Regulations, 1981 (L.N. 103/81)
Cosmetics Products Regulations, 1983 (L.N. 17/83)
Meat (Amendment) Regulations, 1984 (L.N. 37/84)
English Type Sausage Meat (Standards) Regs, 1984 (L.N. 44/84)
Soft Drinks (Amendment) Regulations, 1984 (L.N. 45/84)
Food Hygiene (Amendment) Regulations, 1984 (L.N. 52/84)
Meat Burgers (Standards) Regulations, 1984 (L.N. 53/84)
Ground, Minced & Chopped Meat (Standards) Regs, 1984 (L.N. 59/84)
Food Hygiene (Amendment) Regulations, 1988 (L.N. 50/88)
Dairy Farms (Amendment) Regulations, 1989 (L.N. 98/89)
Bakeries (Use of Fuel) Regulations, 1989 (L.N. 109/89)
Labelling and Presentation of Foodstuffs Regs, 1992 (L.N. 65/92)
Dried Beans (Labelling) Regulations, 1993 (L.N. 82/93)
Labelling and Presentation of Foodstuffs (Amendment) Regulations, 1993 (L.N. 111/93)
Additives in Food Regulations, 1994 (L.N. 89/94)
Soft Drinks (Amendment) Regulations, 1994 (L.N. 90/94)
Colours (Use in Foodstuffs) Regulations, 1995 (L.N. 107/95)
Materials & Articles in Contact with Foodstuffs Regs, 1996 (L.N. 4/96)
Soft Drinks (Amendment) Regulations, 1997 (L.N. 37/97)
Appendix D

Colours (Use in Foodstuffs) (Amendment) Regs, 1997 (L.N. 199/97)
Labelling and Presentation of Foodstuffs (Amendment) Regulations, 1997 (L.N. 201/97)
Sweeteners for Use in Foodstuffs Regulations, 1997 (L.N. 209/97)
Residues in Meat Regulations, 1998 (L.N. 142/98)
Food (Licenses) Regulations, 1998 (L.N. 144/98)
Labelling and Presentation of Foodstuffs (Amendment) Regulations, 1998 (L.N. 145/98)
Veterinary Medicinal Products (Maximum Residue Limits) Regulations, 1998 (L.N. 162/98)
Fruit Juices and Similar Products Mandatory Order ¹ (L.N. 242/98)
Nutrition Labelling Regulations, 1998 (L.N. 247/98)
Infant Formulae and Follow On Formulae Regs, 1998 (L.N. 249/98)
Permitted Food Additives Regulations, 1998 (L.N. 256/98)
Flavourings for Use in Foodstuffs & Source Materials for their Production Regulations, 1998 (L.N. 257/98)
Processed Cereal-based Foods and Other Foods for Infants and Young Children Regulations, 1998 (L.N. 258/98)
Foods Intended for Use in Energy-Restricted Diets for Weight Reduction Regulations, 1999 (L.N. 1/99)
Extraction Solvents for Foodstuffs, 1999 (L.N. 25/99)
Sugar for Human Consumption Order ¹ (L.N. 77/99)
Honey Order ¹ (L.N. 78/99)
Fruit Jams, Jellies, Marmalades and Sweetened Chestnut Puree Order ¹ (L.N. 79/99)

¹ To be issued under Quality Control Act.
NB: Regulations relevant to project appear in bold type.