INVITED REVIEW

The determination of *N*-nitrosamines in food

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Abstract

Introduction *N*-nitrosamines are formed in food as a result of natural chemical interactions, but mainly through food processing activity. Most are potent carcinogens and their determination is therefore of considerable importance. They exist in various chemical forms and have been measured by colorimetric and spectroscopic methods following gas or liquid chromatography or as a total *N*-nitroso group by measurement of chemically released nitric oxide. *Objectives* To provide an overview of the available methods for the analysis of *N*-nitrosamines in food that includes recently developments. *Methods* The literature was reviewed from the discovery of the *N*-nitrosamine problem and the introduction on the *N*-nitroso-specific detector. *Results* The evaluation shows that analytical detection methods for volatile *N*-nitrosamines in food have changed little since the introduction on the *N*-nitroso-specific detector and that research into the occurrence and formation of both non-volatile *N*-nitrosamines and the apparent total *N*-nitroso content (ATNC) have declined. Methods for measuring the apparent total *N*-nitroso content have not been improved significantly in recent years. *Conclusion* Modern sample extraction techniques and mass spectrometric methods for the volatile *N*-nitrosamines have been applied more extensively to water analysis and offer a good opportunity to improve the determination of these carcinogens in food and make the analysis more widely available. Developments in liquid chromatography-mass spectrometry should provide an avenue for renewed interest in non-volatile *N*-nitrosamines, and could help with the identification of novel compounds whose presence is suggested by the high apparent total *N*-nitroso content of some foods.

Introduction

N-nitrosamines are food contaminants that are in most cases strongly carcinogenic (IARC, 1987). The hepatotoxic and carcinogenic properties of *N*-nitrosodimethylamine (NDMA) were first reported by Magee and Barnes (1956) following the discovery that feeding mink with herring meal that had been treated with nitrite caused liver disease (Ender *et al*., 1964). It was found that the nitrite had reacted with dimethylamine naturally present in the fish tissue. The foods that *N*-nitrosamines are usually associated with include beer, nitrite-cured meats, processed cheeses, and fish. In all foods where they occur *N*-nitrosamines are formed by the reaction of nitrosating agents with secondary amines.

NDMA is by far the most frequently encountered member of this group of compounds. Food is a major source of human exposure to *N*-nitrosamines, with others being smoking and endogenous synthesis such as through the reactions of nitrite from vegetables with dietary amines (Bartsch & Spiegelhalder, 1996; Tricker, 1997).

Several authors have reviewed the occurrence of *N*-nitrosamines in food (Crosby, 1976; Crosby & Sawyer, 1976; Fishbein, 1979; Osterdahl, 1991; Tricker & Kubacki, 1992), although improvements in processing procedures have reduced the frequency and level of contamination below that reported 30 years ago. Reviews of analytical methods for *N*-nitrosamines in foods have been published, for example by Walker and Castegnaro (1975), Crosby and

Sawyer (1976), Fan *et al.* (1978), Fishbein (1979), Kubacki (1979), Hotchkiss (1981a, b), Castegnaro and Webb (1983), Scanlan (1984), Sen (1984), Scanlan and Reyes (1985). Many are now outdated as they do not take into account developments in sample preparation, or the advances in mass spectrometry that have been applied to *N*-nitrosamine analysis. A more recent review limited to meats has been published by Rath and Reyes (2009).

N-nitrosamines exist in both volatile and non-volatile forms (Figure 1), and this has a considerable impact on the analytical methods of analysis. The *N*-nitrosamines most frequently encountered in foods are NDMA, *N*-nitrosodiethylamine (NDEA), and *N*-nitrosopyrrolidine (NPYR). The foods and beverages in which detectable levels of nitrosamines are most commonly encountered include cured meats, cheese, fish products, and beer (Mavelle *et al.*, 1991a; Tricker & Kubacki, 1992). Biaudet *et al.* (1994) found NDMA in 427 of 556 food samples (68%) analysed, with the highest level in fish (13.4 mg kg^{-1}). Levels in meat products were much lower (0.04–0.46 mg kg^{-1}).

In pork meats such as bacon and cured hams the nitrite is mostly present as the sodium and/or potassium salt added to preserve the meat from infection by bacteria, in particular the highly toxic *Clostridium botulinum*. The secondary amines present in pork are dimethylamine and pyrrolidine. Pork product manufacturers have an interest in continuing to use nitrite curing as it also gives a favourable colour and

taste to the meat. For the consumer the health risk from *Clostridium* is much greater than that from *N*-nitrosamines and as there is currently no acceptable substitute for nitrite this form of preservation continues. Cured hams therefore continue to contribute to the *N*-nitrosamine intake of consumers, which should be monitored.

In beer the nitrosating reagent is nitrite, which is itself produced by bacterial reduction of nitrate naturally present in the water. The bacteria originate in the yeast used for fermentation, and the formation of nitrite can be reduced significantly by ensuring that the yeast used contains low levels of bacteria. The secondary amine source is dimethylamine produced from gramine present in the barley. In other foods *N*-nitrosamines can be formed from smoking and drying processes, for example in the manufacture of dried milk and some cheeses. *N*-nitrosamines can be formed in water used for food manufacture. Several pathways for this have been identified, including the reactions of some disinfectants (Choi & Valentine, 2002) and some ion exchange resins (Kimoto *et al.*, 1980). Technological steps have been taken to reduce *N*-nitrosamine formation from these sources.

Almost all *N*-nitrosamines have been shown to have carcinogenic activity in experimental animals (Preussmann & Stewart, 1984). The US Environmental Protection Agency (EPA) has classified *N*-nitrosamines as probable carcinogens in humans. In 1997 the EPA estimated the 10^6 lifetime
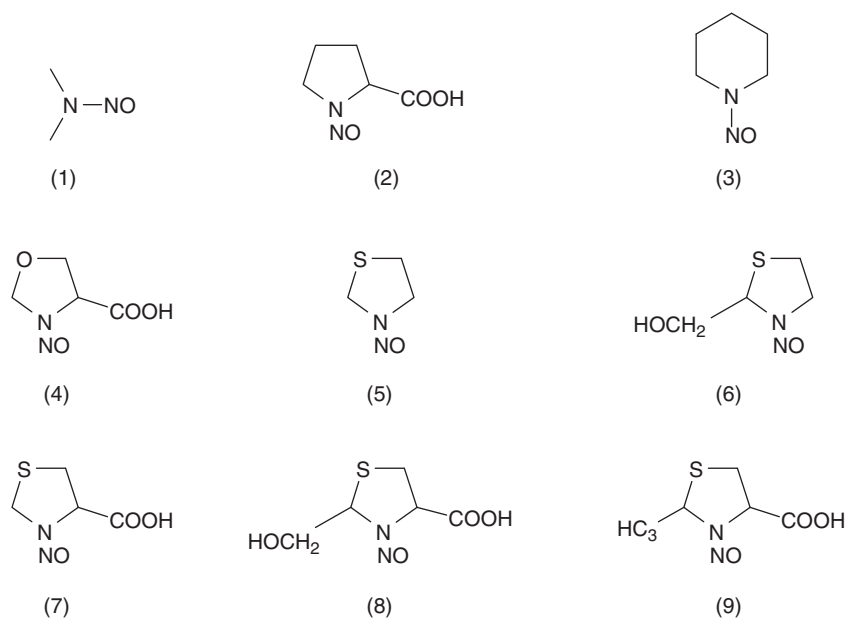

Figure 1 Structures of some volative (1–3) and non-volatile (4–9) *N*-nitrosamines. (1) *N*-nitrosodimethylamine, (2) *N*-nitrosoproline, (3) *N*—nitrosopiperidine, (4) *N*-nitrosooxazolidine–4–carboxylic acid, (5) *N*-nitrosothiazolidine, (6) *N*-nitroso-2-(hydroxymethyl)thiazolidine, (7) *N*-nitrosothiazolidine-4-carboxylic acid, (8) *N*-nitroso-2-(hydroxymethyl)thiazolidine-4-carboxylic acid, (9) *N*-nitrosothiazolidine-4-carboxylic acid.

exposure cancer risk of NDMA at $0.7\,ng\,L^{-1}$ in drinking water (EPA, 1997). The EPA has set maximum admissible concentrations in water for three *N*-nitrosamines (NDMA $7\,ng\,L^{-1}$), *N*-nitrosomethylethylamine ($20\,ng\,L^{-1}$), and NDEA ($2\,ng\,L^{-1}$) (EPA, 2004). Legal limits have been set for *N*-nitrosamines in only a few foods and countries. A limit of $10\,\mu g\,kg^{-1}$ total volatile *N*-nitrosamines have been set for cured meat products in the United States (USDA, 2005), and a limit of 4 and $7\,\mu g\,kg^{-1}$ NDMA in fish and related products was introduced in China in 2005 (USDA, 2005).

The economic impact of the *N*-nitrosamine problem has in the past been very significant as it has affected industries producing beer, whisky, cured meats, and water. Today levels in foods are relatively stable and there is therefore less demand for routine surveillance.

Analytical methods

Extraction

Analytical methods for the determination of *N*-nitrosamines in foods vary distinctly between those for volatile and those for non-volatile *N*-nitrosamines. Volatile *N*-nitrosamines can be extracted from foods with solvents or by distillation and determined by gas chromatography/mass spectroscopy (GC-MS). Distillation can be based on vacuum (Sen & Seaman, 1981; Song & Hu, 1988; Sen *et al.*, 1996), steam (Sen *et al.*, 1997), or mineral oil (Greenfield *et al.*, 1982a, b) techniques. In each case the distillate is collected in a trap or a succession of traps cooled with ice, dry ice, or liquid nitrogen and the *N*-nitrosamines are extracted into a low-boiling solvent, invariably dichloromethane (DCM). The DCM extract is concentrated using a Kuderna–Danish concentrator. This apparatus comprises a round-bottomed flask of 50–250 mL volume fitted with a vertically mounted Snyder fractional distillation column. At the bottom of the flask is a Quickfit joint to which is attached a graduated tapered tube of 5–10 cm in length. The DCM extract half fills the flask and is slowly evaporated to about 5 mL by dipping the tapered tube into a water bath at about $50\,^\circ$C. The distillation needs constant attention to prevent loss of the sample. Finally the tapered tube is removed and the DCM reduced to 0.2–0.5 mL under a stream of nitrogen, often with the use of a 'keeper' such as *iso*-octane. The method is highly efficient for NDMA and has been adopted by the Association of Official Analytical Chemists as Official Methods 982.11, 982.12 984.16, 982.22, and 982.28 (AOAC, 2004).

Mineral oil distillation is preferred to steam distillation, particularly where analysis of higher boiling *N*-nitrosamines

is required. The sample is heated in mineral oil to about $120\,^\circ$C for about 2 h. Scanlan and Reyes (1985) have reviewed such methods.

Distillation and solvent extraction invariably include a Kuderna–Danish concentration step which is challenging in view of the high volatility of NMA. Distillation can be avoided by the use of supercritical fluid extraction (Maxwell *et al.*, 1993; Pensabene & Fiddler, 2000; Fiddler & Pensabene, 1996; Pensabene *et al.*, 1995) where the solvent (liquid carbon dioxide) has a much lower boiling point than NDMA. However this practise has not seen applications in *N*-nitrosamine analysis.

Where the sample is liquid, such as beer or a water extract, it is simpler to use liquid–liquid extraction methods. Beer can be shaken directly with DCM but more usually the sample is adsorbed onto packed columns of an inert support such as Celite (Izquierdo–Pulido *et al.*, 1996; Gloria *et al.*, 1997), Chemelute™ (Mavelle *et al.*, 1991b), or Extrelut™ (Hotchkiss *et al.*, 1981; Baxter *et al.*, 2007; Yurchenko & Molder, 2007). The packing material holds the liquid sample while the eluting solvent is passed through. There is minimal chromatographic separation but the relatively non-polar *N*-nitrosamines are isolated rapidly from the aqueous medium, albeit in quite a large volume of solvent. The extraction therefore has to be followed by Kudernda–Danish concentration as described above. The use of Celite was preferred following a collaborative comparison with similar materials, and has been adopted by the AOAC as Official Method 982.11 (Cutaia, 1982).

Very similar techniques can be used for solid foods, typically cured meats. In the so-called 'dry column procedure' cured meat is dispersed in the solid matrix of diatomaceous earth which is packed into a column and the *N*-nitrosamines eluted with solvent (Pensabene & Fiddler, 1982; Pensabene *et al.*, 1982; Raoul *et al.*, 1997; Yurchenko & Molder, 2007). Yurchenko (2006) studied these methods in detail using solvent mixtures and a range of SPE columns (Extrelut, Florisil, cyano, silica gel, aminopropyl, alumina, and an Extrelut–Florisil combination) for both extraction and clean up. Efficiency and recovery depended on the polarity of the solvent and the sorbent, the nature of the matrix, and the preparation of the sample. Using a well-tested and optimized tandem Extrelut–Florisil SPE method, the best recoveries from meat samples were obtained with use of a 60:40 v/v hexane–DCM mixture to elute *N*-nitrosamines from the Extrelut column, followed by purification using a Florisil cartridge with a 95:5 v/v mixture of DCM and methanol. The recoveries ranged between 74% and 85% with limits of detection of $0.1\,\mu g\,kg^{-1}$ when

chemical ionization (CI) GC-MS was used for separation and detection.

Solid-Phase MicroExtraction (SPME) has recently been used for the determination of volatile *N*-nitrosamines in beer and in some food samples. A fibre needle coated with a bonded organic phase is immersed into the liquid sample or placed in the headspace above solid samples where the *N*-nitrosamines are absorbed. The needle is then retracted and inserted into the injector port of a GC and the *N*-nitrosamines desorbed by rapid heating. Various fibre materials have been used, including polydimethylsiloxane–divinylbenzene (PDMS–DVB), and divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS). Perez *et al.* (2008) have used SPME with mass spectrometric detection to measure NDMA in beer. Andrade *et al.* (2005) used SPME to extract NDMA, NDEA, NPIP, and NPYR from sausages. SPME requires careful optimization of various parameters in order to achieve repeatability, these include the sampling and desorption times and the temperature used to produce equilibration of the *N*-nitrosamines between sample and headspace. It has been shown that equilibrium of *N*-nitrosamines between the sample and the fibre is not always reached, despite the use of lengthy extraction times (Ventanas *et al.*, 2006). Sen *et al.* (1997) reported that the SPME extraction efficiency was too low for most important nitrosamines even with elevated time, temperature, and salt addition.

Chromatography

Following extraction volatile *N*-nitrosamines are readily separated by GC using packed or capillary columns. Because of their non-polar nature and their volatility derivatization is not required, although alkali treatment of packed column stationary phases (typically Carbowax) improves peak shape and hence resolution and sensitivity. A range of capillary column phases of moderate to high polarity have been used with equal success and operational details can be found in the reviews and publications cited. In particular a comparison of different sizes and stationary phase polarities has been made by Borwitzky (1986).

Detection of *N*-nitrosamines following GC can be achieved with nitrogen–phosphorus detectors (Takatsuki & Kikuchi, 1990; Jurado–Sanchez *et al.*, 2007), mass spectrometry (Gough, 1978; Scanlan, 1984), and the nitrosamine-specific chemiluminescence detector or thermal energy analyser (TEA). A comparison of the performance of several detectors was reported by Fine *et al.* (1976c). Descriptions of the mass spectrometric properties of *N*-nitrosamines and

some analytical applications have been described by Libbey and Scanlan (1985), Rainey *et al.* (1978), Webb *et al.* (1983).

Analysis of *N*-nitrosamines was revolutionised by the introduction of the TEA, which offered very high sensitivity and specificity at a time when GC-MS could do so only with difficulty. The TEA instrument comprises a furnace (pyrolyser) operated at a temperature high enough to cleave a nitrosyl radical ($^-$NO) from the *N*-nitrosamine while leaving the remainder of the molecule intact (Fine & Rounbehler, 1975; Fine *et al.*, 1975). The nitric oxide formed passes into a reaction chamber where it is treated with ozone generated from an oxygen supply to produce nitrogen dioxide in an excited state. The nitrogen dioxide passes into a chemiluminescence detector where it releases energy in the form of light at a specific wavelength as it returns to its normal energy state. The light is detected, amplified, and recorded electronically or on a chart recorder. *N*-nitrosamines are in practice the only food components that produce a signal in the TEA when it has been properly set up, and it is highly sensitive.

The lower molecular mass *N*-nitrosamines, and NDMA in particular, have relatively poor mass spectral properties. NDMA has a molecular ion of low mass (*m/z* 74) and potential qualifying ions (*m/z* 42 and 43) that are commonly produced from other compounds. The merits and problems of GC-MS detection of *N*-nitrosamines were discussed by Gough (1978) and by Scanlan (1984). CI techniques are required to provide the optimum sensitivity, and only recently have improvements in MS technology made CI easier to apply. Even so, large sample sizes are required GC-MS detection is more suited to water analysis where concentration by trapping NDMA on to carbon-based columns from large water volumes is feasible. The performance of modern mass spectrometers in *N*-nitrosamine analysis is not significantly different from that of the TEA (Grebel & Suffet, 2007). Yurchenko and Molder (2007) used positive-ion CI with ammonia as reagent gas with selected ion monitoring to determine NDMA in meat products. The limit of detection and the limit of quantitation of *N*-nitrosamine were approximately 0.09 and 0.29 $\mu g\,kg^{-1}$, respectively, with about 85% recovery. NDMA was noted in above 88% of samples, NDEA in 27%, NPYR in 90%, NPIP in 65%, and *N*-nitrosodi-*n*-butylamine in 33% at the mean levels of 0.85, 0.36, 4.14, 0.98, and 0.37 $\mu g\,kg^{-1}$, respectively. The level of total volatile *N*-nitrosamines with the mean of 3.97 $\mu g\,kg^{-1}$ was calculated. The limit of detection and the limit of quantitation of *N*-nitrosamines were approximately 0.09 and 0.29 $\mu g\,kg^{-1}$, respectively, with about 85% recovery. The same authors measured NDMA in beers by a similar technique (Yurchenko & Molder, 2005).

Quantification of volatile *N*-nitrosamines is achieved by the use of calibration graphs enhanced by the inclusion of internal standards. For GC–TEA a synthetic *N*-nitrosamine such as *N*-nitrosodi-*iso*-propylamine is often used whereas when mass spectrometric detection is available stable isotope labelled standards are preferred. Stable isotope labelled internal standards will of course provoke a response in the TEA and are not suitable for use with that detector.

Volatile *N*-nitrosamines have occasionally been measured by high-performance liquid chromatographic (HPLC) techniques more often applied to non-volatile *N*-nitrosamines and described later, namely using denitrosation by hydrobromic acid to produce secondary amines which can be measured by fluorescence detection as their dansyl derivatives (Cardenes *et al.*, 2002a). The detection limit for NDMA in beer was 0.04 $\mu g\,l^{-1}$.

Performance of methods for volatile *N*-nitrosamines.

Analytical methods for the measurement of the major volatile *N*-nitrosamines have in the past been subject of several organized inter–laboratory trials. The results of several trials have been summarized by Scanlan & Reyes (1985). For typical analyses of solid samples such as meats and malt by distillation followed by GC–TEA, and for beers analysed by SPE followed by GC–TEA, detection limits were around 0.1 $\mu g\,kg^{-1}$ and reporting limits 0.2 $\mu g\,kg^{-1}$ (based on signal:noise 3 > 1). Recoveries were typically ranged from 85–98% for samples containing about 10 $\mu g\,kg^{-1}$ *N*-nitrosamine.

Apparent total nitroso content (ATNC)

With the GC column removed the TEA can be used to measure the sum of the nitroso compounds in a food sample by chemically releasing NO from volatile and non-volatile nitroso compounds and passing it directly into the TEA (Walters *et al.*, 1983; Massey *et al.*, 1984a; Pignatelli *et al.*, 1987; Bouchikhi *et al.*, 1989). The *N*-nitroso compound content can be measured against any nitrosamine standard to allow quantification of what has become known as the ATNC. In practice the technique is quite challenging. Several chemical reagents have been evaluated for their efficiency in the denitrosation reaction and a solution of hydrobromic acid in acetic acid has been shown to be the most useful. The sample is usually injected into a heated mixture of solvent (ethyl acetate), hydrobromic acid, and acetic acid. The NO generated is swept by a rapid stream of gas through cold traps and alkali traps to remove reagent

vapour, and passed at a controlled flow rate into the TEA. Several compounds may contribute to a blank response in this apparatus (nitrite, nitrate, *C*- and *S*-nitroso compounds) and it is necessary to inject reagents to remove nitrite, and series of reagent blanks, repeated samples and standards in order to give an accurate result. Addition of acetic acid before adding the hydrobromic acid removes labile NO from nitrite and *C*- or *S* nitroso species. It has been shown to be important to keep the glassware very clean (Massey *et al.*, 1984b), and this includes the removal of denitrosating reagent that might act on the sample too early in the analytical scheme.

Several beneficial refinements have been made to the ATNC method, such as prior removal of nitrite/nitrite ester interferents with sulphamic acid, the use of *n*-propyl acetate as a denitrosation solvent and an increase in the concentration of HBr (Challis *et al.*, 1995). These have improved the water tolerance and the sensitivity of the method, which currently has a detection limit of about 10 $\mu g\,kg^{-1}$ depending on the laboratory and sample type. The methods have been reviewed by Walters (1996) and by Pignatelli and Walters (1996).

The ATNC levels measured in foods invariably exceed the sum of the volatile and known non-volatile *N*-nitrosamines considerably. Part of the difference can be attributed to unknown semi- and non-volatile *N*-nitrosamines.

Method performance for ATNC

A collaborative study of the performance of the ATNC procedure was carried out on behalf of the Commission of Food Chemistry of the International Union of Pure and Applied Chemistry and reported by Castegnaro *et al.* (1987). In other work (Massey *et al.*, 1991) the relative standard deviation for duplicate analyses of 25 samples bacon with an ATNC content of about was about 19%. A bacon containing 6.8 $mg\,kg^{-1}$ *N*-nitroso compounds used as reference material had an RSD of 17%. It has been demonstrated that reproducible results can be obtained for *N*-nitroso compounds as low as 10 $\mu g\,(N\text{-NO})\,kg^{-1}$ for a 1 g sample.

Non-volatile *N*-nitrosamines

A series of non-volatile *N*-nitrosamines can be found, mainly in cured meats (Tricker, 1997). They are mostly nitrosated amino acids or similar compounds and include *N*-nitrosoproline, *N*-nitrosohydroxyproline, *N*-nitrososarcosine, *N*-nitrosothiazolidine, *N*-nitrosothiaozolidine-4-carboxylic acid, *N*-nitrosooxazolidine-4-carboxylic acid,

N-nitroso-5-methyloxazolidine-4-carboxylic acid, and *N*-nitroso-2-(hydroxymethyl)thiazolidine-4-carboxylic acid.

Methods for the determination of non-volatile *N*-nitrosamines have been reviewed by Kubacki (1979). Several applications, mostly related to cured meats, have been published (Fine *et al.*, 1975; Kubacki, 1979; Massey *et al.*, 1984b, 1991; Tricker & Kubacki, 1992).

Certain hydroxylated non-volatile *N*-nitrosamines can be analysed by GC after derivatization with silylating reagents (Eisenbrand *et al.*, 1976; Ohshima & Kawabata, 1979) or in the case of *N*-nitrosoamino acids by methylation of their carboxyl functions to their methyl esters. However, most non-volatile *N*-nitrosamines can only practically be separated by HPLC.

Methods for the use of the TEA as a detector for HPLC were introduced by Fine *et al.* (1976a–c). A special pyrolyser furnace is required to evaporate the solvent and a series of cold traps including a mixture of *iso*-pentane and liquid nitrogen are needed to condense it after the pyrolysis while allowing the NO to pass through. Binary mixtures of solvents with liquid nitrogen boil at specific low temperatures and the mixture containing *iso*-pentane will remove many vapours without freezing nitric oxide. In HPLC-TEA non-aqueous LC solvents are required, which must be free from inorganic buffer to avoid blocking the furnace.

Most *N*-nitrosoamino acids are not believed to be carcinogenic and they have much low toxicity than the volatile *N*-nitrosamines. For this reason, and difficulties in interfacing the TEA with HPLC systems have meant that their study has been little pursued in recent years.

The inability to use reverse phase LC systems for the separation of non-volatile *N*-nitrosamines has been a handicap. It can however be overcome by avoiding the passage of LC solvent into the detector. This is achieved by post-LC column UV irradiation of the sample a matrix of knitted tubing to liberate NO, which can be carried by a gas stream into the TEA (Massey *et al.*, 1991). Alternatively the HPLC eluent can be mixed with a solution of naphthylenediamine and sulphanilamide, known as the Greiss reagent, which turns a pink colour to a degree equivalent to the quantity of NO present, which can be quantified by spectroscopy (Conboy & Hotchkiss, 1989; Bellec *et al.*, 1996).

In an alternative approach an analysis can be made of the secondary amine content of the sample both before and after denitrosation by UV irradiation or treatment with hydrobromic acid in acetic acid (Cardenes *et al.*, 2002a, b). The secondary amines are readily determined by LC with fluorescence detection after formation of their dansyl derivatives.

Method performance for non-volatile *N*-nitrosamines

In an early collaborative trial reported by Walker & Castegnaro (1975) eight laboratories provided data for the analysis of pork luncheon meat spiked with NDMA, NDEA, NDBA, and NPYR at about 6 µg kg^{-1}. The mean values reported for each of the *N*-nitrosamines were between 4.6 and 4.9 µg kg^{-1}. Average within-lab standard deviations ranged from 0.7 µg kg^{-1} (NDEA) to 1.8 µg kg^{-1} (NPYR) with coefficients of variation close to 20% except for NYPR (38%). Average between-lab standard deviations were close to 2 µg kg^{-1} except for NPYR (2.8 µg kg^{-1}) with coefficients of variation close to 50%.

For all methods of *N*-nitrosamine analysis measures must be taken to prevent false-positive signals, particularly those resulting from nitrozation of amines during the extraction and work-up (Hansen *et al.*, 1979; Webb & Gough, 1979; Walker & Castegnaro, 1980; Eisenbrand *et al.*, 1983; Walters *et al.*, 1984). Preventative methods include the addition of ammonium sulphamate to remove nitrite, and the inclusion of a nitrosatable secondary amine, such as morpholine, that is not found in food, to demonstrate any artefactual *N*-nitrosamine formation. Antioxidants such as α-tocopherol, propyl gallate, and ascorbic acid also inhibit *N*-nitrosamine formation.

Confirmatory methods

The identity of suspect *N*-nitrosamines detected by the TEA may be confirmed to some degree by irradiating the sample with UV light (Kimoto & Fiddler, 1982; Budeveska *et al.*, 1986). This decomposes the *N*-nitrosamine and the TEA response disappears. However, this is not a very positive form of identity confirmation. An alternative confirmatory procedure involves oxidation of the *N*-nitrosamine to the equivalent nitramine with hydrogen peroxide and detection of this by GC (Althorpe *et al.*, 1970; Sen, 1970). For GC-based methods where sufficient sensitivity is available GC-MS is the preferred confirmatory method.

Conclusions

There is scope for considerably more research into *N*-nitrosamine formation and occurrence, and the application of newer chromatographic techniques, the most promising of which probably being two-dimensional gas chromatography, and hydrophilic interaction chromatography, that are only just beginning to be applied in this area (Pan *et al.*, 2004) and could prove to be of great benefit in the separation of volatile and non-volatile nitrosamines, respectively.

GC-MS methods based on SPME are becoming established for water matrices and their extension for the determination of volatile *N*-nitrosamines in solid foods such as meats is anticipated. Application of modern HPLC-MS methods to the identification of novel non-volatile *N*-nitrosamines could lead to identification of further components of the ATNC fraction. A major advantage of HPLC-MS methods is their compatibility with mobile phases that contain a high proportion of water. Time of Flight mass spectrometers have sufficient sensitivity to provide empirical formulae of low levels of food, components from which structural elucidation is possible, and tandem (LC-MS/MS) techniques provide complementary structural information or additionally very high sensitivity. The use of such methods could also have a role in the determination of *N*-nitrosamines in food and their metabolites in body fluids.

No significant improvements have been made to the methods for ATNC in many years. Advances are overdue in the updating of the denitrosation systems, in improvement to the means of the transfer of NO into the TEA, or the use of alternative detectors such NO traps or Griess-type reagents.

Finally, acceptable standards of quality assurance and control have changed somewhat since the development and testing of the TEA-based methods in the 1980s. Most researchers have published details of successful attempts to reduce artefactual formation of *N*-nitrosamines during sample processing, but the use of reference materials (even in-house materials) and regular use of blank materials and QC standards has rarely been reported for food analyses. The situation for the analysis of water for *N*-nitrosamines by GC-MS is considerably better, and these modern approaches to quality and validation must soon be applied to all other areas of this fascinating topic.

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