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Determining preservatives in meat products by flow injection analysis (FIA): A review

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Abstract

Various preservatives are added to meat products to extend their shelf-life stability and enhance their safety. Determining preservatives is essential for purposes of legislation and consumer safety. The analytical methodologies based on flow injection analysis (FIA) can be highlighted among those available. FIA methodologies offer attractive advantages compared with other procedures, including versatility, precision, low cost, speed and ease of automation. This review considers the present status of published FIA methodology available for the determination of preservatives in meat products. The techniques are described with their application to different preservatives (nitrates and nitrites, sulfites, sorbates, benzoates and p-hydroxybenzoate esters), emphasizing aspects related to extraction, separation, detection and quantification procedures in meat matrices.

Key words: Meat products, additives, preservatives, nitrates and nitrites, sulfites, sorbates, benzoates and p-hydroxybenzoate esters, flow injection analysis.

Introduction

Meat and meat products are highly perishable because they contain significant amounts of the nutrients needed for micro-organism growth. The development of bacteria, yeast and molds is one of the main agents responsible for the deterioration of these food products, limiting their stability with important effects on the product quality and safety levels, and relevant economic implications. Various preservation procedures are used to protect meat from microbial spoilage, including those based on additives known as preservatives. These are chemical compounds added to foodstuffs which prolong their shelf-life by protecting them (by prevention or inhibition) from deterioration caused by microorganisms and thereby enhance food safety.

The control and regulation of the use of preservatives in meat products are very important because they are effective and ensure consumer safety. The acceptable daily intake (ADI) for preservatives has been calculated, just as for other food additives, since if they are consumed in inappropriate quantities they may have adverse effects on human health. This is why legislation on the use of additives is needed to establish specific conditions for their use. Food additive legislation adopted by the European Union is included in several European Parliament and Council Directives (Directives 95/2/EC and 2006/52/EC). The authorized preservatives for meat products are shown in Table I. To be able to evaluate whether they are being used in the specified quantities and/or foods , it is essential to have appropriate analytical methods available.

Numerous methodologies for determining preservatives in foodstuffs are cited in the relevant literature, including specific references to meat products. They are based on separation, identification and quantification processes using a wide variety of spectrophotometric, chromatographic and electrophoretic techniques etc. (Fazio and

Warner 1990; Karovicová and Simko 2000; Wood et al. 2004; Ruiter and Bergwerff 2005; Jiménez Colmenero and Blazquez 2007). Among the methodologies, those based on flow injection analysis (FIA) are of particular interest and have developed significantly in recent years because of the many advantages they offer. FIA is a very versatile, flexible, economical technique, suitable for the fast, routine analysis of a large number of samples with high precision. Affordably priced and easy to use, it requires minimum intervention of the operator and a lower consumption of samples and reactants. This is especially important when working with toxic or expensive reactants. It can be installed on-line and is compatible with a large number of detectors (potentiometric, amperometric, spectrophotometric, fluorimetric, etc.). This methodology responds to growing demands for the mechanization and automation of analysis made by many laboratories faced with the increasing number of routine analyses. This, along with its advantageous cost/efficiency relationship, favors the use of this method over the alternative procedure for determining additives (Valcarcel and Luque de Castro 1987; Sullivan et al. 1990; Ferreira et al. 1996; Pinho et al. 1998; Kazemzadeh and Ensafi 2001; Andrade et al. 2003; Frenzel et al. 2004, Penteado et al. 2005).

Early studies of FIA focused on the determination of contaminants in water (a matrix generally easy to work with and with little interference except in waste water), but by the mid-80s the applications of FIA had been extended to other fields (food, biomedicine, control of industrial processes etc.), in more complex matrices (Valcarcel and Luque de Castro 1987). Although FIA has been trialed to determine some preservatives in meat products no in-depth critical analysis has been made of the use of this methodology and the possibilities it offers, as far as the authors are aware. This review considers the current status of the available published flow injection analysis methodology for the determination of authorized preservatives in meat products. To do this, different aspects are analyzed, related to extraction, separation, detection and quantification procedures in complex muscle matrices.

Brief description of FIA

The original concept of FIA was established by Ruzicka and Hansen in 1975, and has been continuously modified since then. Flow injection analysis is based on the injection of a liquid sample into a moving, non-segmented continuous carrier stream of a suitable liquid. The injected sample forms a zone which is then transported towards a detector that continuously records phisical parameter such as the absorbance, electrode potential, or other physical parameter, changing as the sample material passes through the flow cell. The FIA response curve (FIAgram) is the result of two kinetic processes: the physical process of dispersion of the sample zone within the carrier stream and the chemical process of formation of a chemical species. These two processes occur simultaneously and together with the dynamic characteristics of the detector, they yield the FIA response curve.

The simplest FIA system (see Figure 1) consists of a tube through which the carrier stream moves towards the flow-through detector. Briefly, it consists of the following basic elements:

- **Propulsion system.** This is generally a peristaltic pump with a variable number of channels, depending on the moving or reactive (carrier) phases which intervene in the reaction. It provides a constant flow of a carrier solution.
- **Injection system.** This is normally a low pressure Rheodyne valve which allows a small volume of the sample, usually measured in micro liters, to be inserted into the flow of the carrier solution. The use of valves with various ports allows the simultaneous determination of various analytes.
- Reaction coils and complementary components. The most frequently used reactors are tubes made from various materials such as polytetrafluorethylene or plastic tubing of different sizes and diameters (e.g. ID 0.8 mm), which can be coiled, knitted or knotted. The purpose of this geometric deformation is to decrease the zone dispersion. These coils can be thermostatized. Sometimes the system can also have other integrated elements in series or in parallel (Figures 2 and 3) such as columns (e.g. to cause chemical reactions as with cadmium for the transformation of nitrates to nitrites or to immobilize reactants), or gas diffusion cells (e.g. for separating SO₂ or NO₂).
- **Detection systems.** A wide variety can be used, including potentiometric, amperometric, spectrophotometric, fluorometric, etc.
- **Data collector system.** This is normally carried out from a PC linked to the system or to the detector, with signal integration software.

Nitrites and nitrates

Nitrites and nitrates are used in processed meat because of their effect on its organoleptic characteristics (color stabilization, flavor development), oxidative stability of lipids and inhibition of pathogenous micro-organisms such as *Clostridium botulinum* or *Listeria monocytogenes* (Cassens et al. 1979; Cassens 1997; EFSA 2003). Although the positive effect of nitrite on human health has been indicated (protective role of nitric oxide), the interest in nitrite and its reaction is mainly centered on the potential formation of nitrosamines (on reaction with secondary amines), compounds with teratogenic, mutagenic and carcinogenic effects, highly dangerous for human health (Cassens 1997). For this reason and for some time now, the possibility has been put forward of reducing or even avoiding the use of these additives in the food industry.

In the EU, potassium and sodium nitrite and nitrate are authorized for use in different meat products, and maximum amounts (150 mg kg-1 for nitrite and 300 mg kg-1 for nitrate) are established for all products as well as maximum residual levels for some of them (Directives 95/2/EC and 2006/52/EC). Current regulations on the use of nitrite and nitrate in the US vary depending on the curing method used and the product to be cured. For comminuted products, the maximum ingoing nitrite and nitrate limits are 156 mg kg-1 and 1718 mg kg-1, respectively. For immersion-cured and massaged or pumped products those limits are 200 mg kg-1 and 700 mg kg-1, respectively. In dry cured products nitrite is limited to 625 mg kg-1 and nitrate to 2187 mg kg-1 (USDA 2007). The ADI for nitrites, as nitrite ion, is 0.07 mg kg-1 body weight, and 3.7 mg kg-1 for nitrates, as nitrate ion (FAO/WHO 2007a, b).

Different methodologies have been used to determine nitrates and nitrites, including ion chromatography, capillary electrophoresis, differential pulse voltammetry, enzymatic reduction and electrochemistry (biosensors with amperometric monitoring among others). For these, different detection systems based on UV, mass spectroscopy, fluorescence, chemiluminiscence or electrochemical detection have been used (Usher and Telling 1975; Wood et al. 2004; Ruiter and Bergwerff 2005; Jiménez Colmenero and Blazquez 2007). Among these methodologies a prominent position is occupied by those based on flow analysis injection with their unquestionable advantages, which have been applied in numerous meat products (see Table II).

Generally speaking, the determination of nitrates and nitrites by FIA can be divided into two phases, as is also the case with other preservatives. The first phase is the extraction process of these compounds from the meat matrix and the other is its evaluation *per se* using FIA methodology. Both these phases are analyzed below.

Nitrate and nitrite extraction

The extraction of nitrate and nitrite from meat products is the most critical in terms of obtaining adequate recoveries for the nitrates and nitrites and to reduce possible interferences. When nitrite is added to a biological material such as meat products, it reacts with various components present naturally in the matrix, which means that the levels of nitrite detectable analytically (known as residual) necessarily vary. This means that the levels of residual nitrite fall during the different stages of processing, storage, preparation and consumption depending on various factors related to the type of product and the conditions of each of the phases mentioned above. (Cassens et al. 1979; Cassens 1997; EFSA 2003). Different procedures have been used in the extraction of nitrate and nitrite from foods including processed meats.

Just as with other analytical methods, most of the nitrite and nitrate extraction processes by FIA determination use hot water and different systems for blending or homogenizing the sample and filtration (see Table II). An extraction system for cured meat based on microwave cooking of the samples following cold extraction (water and NH₄Cl at different pH) in a nitrogen atmosphere has been reported by van Staden and Makhafola (1996).

Some compounds present in meat products (ascorbic acid, fat, protein, etc.) which can interfere in the determination of nitrates and nitrites, may be present in the extract. This is why it has been emphasized that purification or clarification steps need to be followed. Several clearing techniques have been used for the analysis of nitrite and nitrate in food especially in meat products. These may be based on de-proteinisation and clarifying agents using reagents such as potassium aluminum sulfate, borax (sodium tetraborate anhidra), mercuric chloride, zinc sulfate, or potassium hexacyanoferrate (Carrez I) plus zinc acetate (Carrez II), the most commonly used clarifying agents for aqueous extracts of meat samples (see Table II). Other clearing techniques may also be used such as active carbon or gel filtration on Sephadex G25 - this last only used for

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beverages (Torro et al. 1998). Some of these reagents have been used to precipitate proteins from meat extract and also to fix the pH between 6.0 and 6.5, to avoid nitrate and nitrite loss. One of the major criticisms leveled at the use of Carrez is that the pH of the final solution (approximately pH 5) tends to promote nitrite loss. However, this deproteinization procedure of samples was also complicated because it caused cloudy filtrates and as a result serious experimental errors (Usher and Telling 1975).

Measurements of nitrite

In the FIA analytical methods the extracted samples are generally injected through the injection system valve into the corresponding carrier streams (see Figure 2; see Table II); this is generally a reagent composed of ammonium chloride and EDTA (van Staden and Makhafola 1996; Oliveira et al. 2004; Ruiz-Capillas et al. 2006) or ammonium chloride, sodium tetraborate and EDTA (Pinho et al. 1996; Ferreira et al. 1996) or a buffer 7.5 (Ruiz-Capillas et al. 2007). These masking and buffering reagents draw the injected extracted solution into the coil of different lengths; then it is mixed directly with the color forming reagent (in most cases, since most of the methods used for nitrite determination by FIA in meat products are colorimetric) in a second reaction coil (see Figure 2; see Table II). The color forming reagents are mainly those used in the traditional diazo-coupling reaction (the Griess reaction), generally composed of sulphanilamide, (N-(1-naphthyl)ethylenediamine dihydrochloride - NED) and phosphoric acid (Ferreira et al. 1996; Pinho et al. 1996; van Staden and Makhafola 1996; Ruiz-Capillas et al. 2006, 2007) or other acids such as HCl (Oliveira et al. 2004; Frensel et al. 2004). These procedures are based on the well-known ability of nitrite to convert aromatic amines (sulphanilamide) into diazonium ions, which are coupled to another aromatic compound (NED) in order to produce an azo-dyamine which is detected spectrophotometrically at 520-540 nm. This FIA system based on the Griess reaction has been also used for the determination of protein-bound nitrite in frankfurter and dry sausages (Ruiz-Capillas et al. 2007).

Although the Griess reaction presents important advantages, including excellent sensitivity, it also has certain limitations in relation to the control requirements of the reaction conditions, stability of the reactants, interferences or the use of some reactants which present carcinogenic effects. Many of these problems were also reported when the FIA version of the Griess reaction was applied to meat matrices. E.g. van Staden

and Makhafola (1996) reported that for certain samples, mainly from an acidic origin (cured meat products), the experimental nitrite value obtained was lower than the expected value. To solve these problems it has been recommended that the nitrite samples should always be kept under nitrogen atmosphere during preparation and they must always be analyzed immediately after preparation or stored under nitrogen atmosphere. The nitrite solution was also standardized against a permanganate solution treated with a few drops of chloroform and kept in the refrigerator (van Staden and Makhafola 1996). SO₂ can also interfere with nitrite determination (Ahmed et al. 1996). Haghighi and Tavassoli (2002) showed the importance of the dye instability, so that the color reaction must be time controlled as it is affected by numerous oxidation reactions.

Over recent years, and as the result of these drawbacks, a number of other amines have been proposed as potential reactants for nitrite and potential coupling agents applied to the analysis of food extracts by FIA spectrophotometric determination (see Table II). For example, 3-nitroaniline, with NED, has been reported as a spectrophotometric reagent (color-forming reagent) for nitrite and nitrate determination in beef and pork sausages (Ahmed et al. 1996). Safranine O (3,7-diamino-2,8-dimethyl-5phenylphenazinium chloride) has also been reported to form diazonium salts in an acidic solution which causes the reddish-orange dye color of the solution to change to blue in acidic media and which absorbed at 520 nm has been used for flow injection spectrophotometric determination of nitrite in beef and commercial sausages (see Table II). The reaction of safranine with the nitrite decreases the color of the dye instead of its appearance as in the case of diazodye (Kazemzadeh and Ensafi 2001; Penteado et al. 2005).

Andrade et al. (2003) also reported the development and application of FIA based on spectrophotometric methods for the determination of nitrite in foodstuffs (vegetables and meat products). This is based on the formation of FeSCNNO⁺ in the flow injection system, with transient absorbance signal at 460 nm (see Table II). The method is based on the reduction of the nitrite to NO in a sulfuric acid medium and the reaction of nitric oxide with ferrous and thiocyanate ions forming FeSCNNO⁺.

Oxidation/reduction reaction has been also used in the determination of nitrites. Ensafi and Kazemzadeh (1999) proposed a flow injection method with spectrophotometric

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detection for determination of nitrite in beef samples based on the catalytic effect of nitrite on the oxidation of gallocyanine by bromate in acidic media and the decrease in absorbance of the system at 530 nm (see Table II). Sen et al. (1994) also developed a FIA method based on nitrite reduction with potassium iodine followed by chemiluminescence detection of the liberated NO using a thermal energy analyzer for the determination of nitrites in food (cured meat) and other biological materials.

Torró et al. (1998) developed a FIA method (tested on vienna sausages) that involved the photochemical reduction of nitrate in a photo reactor consisting of a low pressure mercury lamp. The nitrite produced is determined indirectly with the I_3/I^{-} system using a flow-cell with two platinum (Pt) electrodes polarized at 100 mV (see Table II).

Haghighi and Tavassoli (2002) reported a flow injection method on the basis of gas phase molecular absorption for the determination of nitrite in meat sausages and mortadella and aqueous solutions (see Table II). The sample solution is injected into a stream of water which is then reacted with a stream of hydrochloric acid. The gaseous products (NO, NO₂, HNO₂ and NOCl) are separated from the liquid stream by the home-made gas liquid separator and they are swept by the carrier O₂ gas into a homemade flow-through cell which has been positioned in the space of the cell compartment of a UV/VIS spectrophotometer. The transient absorbance of the gaseous phase is measured at 205 nm. The interference of anions in the methods proposed by Haghighi and Tavassoli (2002) are overcome adding silver and sodium hydroxide solutions as the precipitating agent. This method is less sensitive than the Griess methods but there are also fewer limitations than in the Griess method.

Frenzel et al. (2004) use a system of gas diffusion membrane-based optical flow through optical sensor for the determination of nitrite in waste waters and meat extracts (see Table II), using the modified Griess reaction where sulfuric acid was used as a releasing agent to set HNO_2 and NO_2 free from the nitrite solutions. The color reagent was prepared by a dissolution of sulfanilamide, *N*-(1-naphthyl)-ethylenediamine dihydrochloride and concentrated hydrochloric acid in water. The main reason for developing the proposed sensor was the inherent selectivity enhancement due to analyte separation by the gas-diffusion process. Only a few analytes are sufficiently volatile to pass through the membrane and they are effectively separated from the sample matrix.

Species known to interfere in the Griess reaction are therefore almost entirely excluded. In particular, colored and turbid samples can be analyzed without problems and interferences as extreme pH values or the presence of high salt content in the samples are eliminated so that the proposed sensor is almost nitrite specific. However, the relatively low sensitivity of the developed sensor (compared to the common spectrophotometric method (ISO 1996) limits its applicability (Frenzel et al. 2004).

Measurements of nitrates and nitrites

Although initially FIA was used only for nitrite determination, nitrate determination was also developed later. Since for nitrate analysis the previous reduction of nitrate to nitrite is required, FIA methodology included a reduction system. This means that in its application in meat products the nitrate is reduced on-line to nitrite in a cadmium column which is then treated with azo dye reagent and the absorbance due to the sum of nitrite and nitrate is measured: nitrate is determined from the difference in absorbance values (see Figure 2) (Ruiz-Capillas et al. 2006, 2007).

The simultaneous determination of nitrates and nitrites has been trialed since this substantially reduces analysis times. Ahmed et al. (1996) determined nitrites and nitrates (beef and pork sausages) simultaneously using a selector valve to split the sample into two streams after it was injected into the carrier stream. The quantification was based on the 3-nitroaniline+NED color reaction (see Table II). For different meat products Ferreira et al. (1996) and Pinho et al. (1998) also determined nitrate and nitrite simultaneously. The test was based on the splitting of the flow after injection and subsequent confluence of the flow before it reached the detector, allowing the reduction of nitrate to nitrite in part of the sample plug in an on-line copper cadmium redactor column. Since each channel has a different residence time two peaks were obtained for nitrite and for nitrite plus nitrate. The spectrophotometric determination was made after a diazotization coupling reaction. A similar system for simultaneous determination of nitrite and nitrate in various beef samples used flow injection with spectrophotometric detection based on a oxidation system where the nitrate is reduced to nitrite by using two sequential columns: copper and copper-coated cadmium (Ensafi and Kazemzadeh 1999). Kazemzadeh and Ensafi (2001) reported similar procedure for the simultaneous determination of nitrate and nitrite but based on the reaction of nitrite with safranine O (see Table II). On the other hand, Oliveira et al. (2004) reported a simultaneous

determination of nitrate and nitrite in cured meat by sequential injection analysis, based on the Griess reaction.

Sulfites

Sulfites are used as preservatives in food and beverages to prevent oxidation and bacterial growth. In meat, sulfites also help to give a bright color. Appearance (colour) is one of the prime factors motivating consumer choice. In the EU the maximum permitted amount of added sulfite agents in different meat products is 450 mg kg-1, expressed as SO₂ (Directives 95/2/EC and 2006/52/EC). These compounds are not permitted for use in meat in the USA. Sulfites have been associated with allergic reactions and food intolerance symptoms. They are also known to degrade thiamine (vitamin B1), of which meat is a good source. The ADI for sulfite (expressed as SO₂) is 0.7 mg kg-1 body weight (FAO/WHO 2007c).

There are many methods for analyzing sulfites in beverages and foods. These procedures involve titrimetry, electrochemistry, fluorimetry, chemiluminesence spectrometry, colorimetry, gas-liquid chromatography, high preformed liquid chromatography, etc. (Fazio and Warner 1990; Karovicová and Simko 2000; Wood et al. 2004; Ruiter and Bergwerff 2005; Jiménez Colmenero and Blazquez 2007). The most commonly used techniques are based on the classic Monier-Williams method. Although this method demonstrates sufficient sensitivity, it also presents certain limitations because of the long analysis time (with important repercussions on the analytical capacity of the laboratory) and the extreme control needed in some of the analytical phases (e.g. N_2 stream adjustment).

FIA procedures have been extensively researched for determining sulfites in beverages and foods, in an attempt to overcome these limitations and reduce the time and effort needed for those procedures. Most FIA procedures have been developed for liquid foods, with far fewer for solid foods and especially for muscle foods (see Table III). It has been reported that the FIA procedure is a more rapid, accurate and precise method for the determination of total sulfite in food than the Monier-Williams procedure (Sullivan et al. 1986, 1990). Sulfite determination by FIA generally consists of two phases: one related to the extraction process of the sulfiting agent from the food and the other related to evaluating it.

Sulfite extraction

When sulfiting agents are added to foods such as fish or meat products they can combine with some of their components (aldehydes, proteins etc.), so that they may be present as free, reversibly bound (probably as hydrosulphonate adducts) and irreversibly bound (Wedzicha and Mountfort 1991; Pizzoferrato et al. 1998). The relative presence of each of these depends on factors linked to composition, processing and conservation. This is why the determination of sulfites in complex food matrices such as meat products requires the application of some procedure for removing and recovering the sulfites (free and reversibly bound both together called total sulfites). Since substantial loss of sulfites can occur during extraction, different alternative extraction conditions (distillation, acidic, alkaline extractant, etc.) have been tried, to obtain free and bound sulfite fractions. For analysis using FIA various extraction media have been trialed (including solutions containing acetone, formaldehyde and ethanol), but the most efficient extraction procedure in muscle foods for FIA analysis includes pH extraction with a sodium tetrachloromercurate (TCM) solution (Sullivan et al. 1986, 1990; AOAC 2005) (see Table III). TCM helps to release the sulfite from solid foods and stabilize it forming a stable sulfite-mercuric complex, providing excellent sulfite recovery (Sullivan et al. 1986; Ruiter and Bergwerff 2005).

Measurements of sulfites: separation/detection systems.

Afterwards, the extracting solution obtained from muscle food matrices (meat and fish) containing the sulfiting agents is injected into the FIA system to proceed to the separation of the SO_2 followed by the corresponding detection process (see Figure 3).

The separation phase in these matrices is based on the use of a gas diffusion process (Sullivan et al. 1986; AOAC 2005). For determination of total sulfites previous to this separation in the gas diffusion system a hydrolysis treatment is applied with NaOH aimed at the release of most of the bound sulfites from the extract solution which is complete above pH 8 (Kim and Kim 1986; Sullivan et al. 1990; AOAC 2005) (see Figure 3).

The separation of SO_2 itself is carried out in the gas diffusion cell (see Figure 3). Two liquid streams converge in this cell: the first is a donor stream which transports a strong acidic donor solution intended to provoke the release of the SO_2 present in the test solution. Although other donor reagents may be used in gas diffusion systems, sulfuric acid is what is used in muscle based food (Sullivan et al. 1986, 1990; AOAC 2005). This choice is closely linked to the detection system. The second is an acceptor stream (indicator which collects the released SO_2), a solution containing an acid-base indicator separated by a permeable membrane (generally made of teflon). The SO₂ released from the donor diffuses through the membrane and is dissolved into the acceptor solution. The membrane is not only necessary to separate the strong acidic donor solution from the acceptor solution but also forms a barrier for potential interferences in the sample. Different colorimetric reagents (iodine, bromocresol green etc.) can be found among the acceptor solutions used, although in the determination of SO_2 by FIA in solid matrices (muscle based food) a malachite green solution is used (Sullivan et al. 1986, 1990; AOAC 2005) (see Table III). The degree of discoloration of malachite green is proportional to the amount of sulfite in the test solution and it is measured spectrophotometrically at 615 nm. Spectrophotometric detection seems more appropriate as it is more robust and it is usually available in routine control laboratories.

The possible interferences in the determination of SO_2 by FIA with a gas diffusion unit are related to the presence of compounds able to pass through the membrane, including nitrite, sulfide and cyanide (Frenzel and Hillman 1995). Although these interferences have been established in beverages, their incidence is plausible under any circumstances in solid matrices such as meat products.

Sorbates, benzoates and p-hydroxybenzoate esters.

Sorbic acid, benzoic acid and the methyl and ethyl esters of 4-hydroxybenzoic acid (parabens) or their (Na, K, or Ca) salts are organic acids used as meat product preservatives (see Table I). In the EU they are allowed in the surface treatment and jelly coating of many processed meats, used singly or in combination (sorbates, benzoates and parabens in concentrations ranging from 1000 mg kg-1 to *quantum satis*) and in pâtés (sorbates and parabens used singly or in combination), up to a maximum level of 1000 mg kg-1. (Directives 95/2/EC and 2006/52/EC). The acceptable daily intake is 25

mg kg-1 for sorbic acid (FAO/WHO 2007d) and 5 mg kg-1 body weight for benzoic acid (FAO/WHO 2007e). The EFSA has established a full-group ADI of 10 mg kg-1 body weight for the sum of methyl and ethyl p-hydroxybenzoic acid and esters and their sodium salts (Directive 2006/52/EC).

Given that these compounds present strong UV absorption, the method most commonly used to determine organic preservatives in foods is spectrophotometry. Other methods have also been reported including thin-layer chromatography, gas chromatography, high performance liquid chromatography and capillary electrophoresis among others (Karovicová and Simko 2000; Wood et al. 2004; Ruiter and Bergwerff 2005; Jiménez Colmenero and Blazquez 2007).

The application of FIA method for the determination of sorbic acid, benzoic acid and parabens in meat products has not been found in the literature. A sorbic acid determination method by FIA in wine has been proposed (Molina et al. 1999). The advantages offered by the traditional spectrophotometric methods in terms of simplicity and specificity may help to understand the lack of interest in the application of other methodologies, including FIA, to the determination of these compounds in foods. There is no methodological reason which limits the application of FIA in the determination of these preservatives in meat products. The procedure requires, just as for other methods, an extraction process (for the extraction of the compound and, where relevant, the elimination of interferences related to the nature of the matrix) before it is introduced into the equipment, where the color reaction is developed and later detection, both visible and UV, takes place. In this case a basic advantage of the FIA system is the possibility of automation, which would mean an increase in the volume of samples analyzed and reduced handling.

CONCLUSIONS

The application of FIA to determine preservatives in meat is an important alternative to more conventional methods and presents several advantagesn in routine laboratory analysis for official bodies and industry. FIA is a method which allows a simple, precise, fast determination of small amounts of sulfite with low reagent consumption, small volumes of samples required and the use of different detection systems. However, more studies are necessary in order to develop new FIA procedures for the

determination of other preservatives present in meat and meat products (e.g. sorbates, benzoates etc.). It is also necessary to improve the present procedure in order to reduce the quantity of toxic reagent that is used in some of them. More studies of the extraction and cleaning procedures will be necessary for the application of FIA in complex matrices such as muscle based foods. Therefore preservative recovery should be improved and the reduction of possible interference associated with both natural matrix components and other food additives should be studied. Studies regarding the automatization of all the procedures must also be carried out, as well as reviewing the stabilising reagent for the extraction solution and its conservation to avoid substantial losses of preservatives. In depth studies will also be necessary to apply FIA procedures to new products (including meat based functional foods), which have been developed using new non-meat ingredients (e.g. from plant and marine sources).

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 Table I.- Food Additives approved by the European Union (1) for use in meat products (E-Number list)

Groups	E numbers	Names	
Sorbates (Sa)	E-200	Sorbic acid	1
	E-202	Potassium sorbate	1
	E-203	Calcium sorbate	1
Benzoates	E-210	Benzoic acid	1
(Ba)	E-211	Sodium benzoate	1
	E-212	Potassium benzoate]
	E-213	Calcium benzoate]
Paraben (PHB)	E-214	Ethyl-p-hydroxybenzoate	1
	E-215	Sodium ethyl-p-hydroxybenzoate	1
	E-216	Propyl p-hydroxybenzoate ¹	1
	E-217	Sodium propyl p-hydroxybenzoate ¹]
	E-218	Methyl-p-hydroxybenzoate	
	E-219	Sodium methyl-p-hydroxybenzoate	
Sulphites (SO ₂)	E-220	Sulphur dioxide	
	E-221	Sodium sulphite	
	E-222	Sodium hydrogen sulphite	
	E-223	Sodium metabisulphite	
	E-224	Potassium metabisulphite	
	E-226	Calcium sulphite	
	E-227	Calcium hydrogen sulphite	
	E-228	Potassium hydrogen sulphite	
Nitrates (NO ₃) &	E-249	Potassium nitrite	
Nitrites (NO ₂)	E-250	Sodium nitrite	
	E-251	Sodium nitrate	
	E-252	Potassium nitrate	

Food additive legislation: European Parliament and Council Directives (Directives 95/2/EC and 2006/52/EC)

I able II Nitrate and nitrite determination in meat and meat products by flow injection analysis	e II Nitrate and nitrite determination in meat and meat products by	y flow injection analysis
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	Sample	Extraction/Clarification	Carrier	Reduction NO ₃ to NO ₂	Colorimetric reagent	Detection system	Ref
Nitrite	Cured meat	Homogenised with hot water and NaOH solution, transferred flask to a bath (50-60°C) and occasional shaking, then addition of zinc acetate and filter.			Iodine	Chemiluminescence	Sen et al. 1994
Nitrate and Nitrite (simultaneous)	Ham, sausages, smoked pork sausages and smoked ham	Homogenised with hot water and followed by purification and filtration by addition of disodium tetraborato and Carrez reagent (potassium hexacyanoferrate and zinc acetate)	Ammonium chloride + sodium tetraborate + EDTA	Copper - cadmium reductor column	Sulphanilamide NED Phosphoric acid	Spectrophotometric 530 nm	Ferreira et al. 1996
Nitrite	Cured meat samples	Cooking of the samples in a microawave oven and then cold extraction under N_2 atmosphere with water and + NH_4Cl	Ammonium chloride + EDTA		Sulphanilamide NED Phosphoric acid	Spectrophotometric 540 nm	van Staden and Makhafola 1996
Nitrate and Nitrite (simultaneous)	Beef and pork sausages	Sample was mixed with sand and homogenised in a mortar and digested in a bath (100°C) during 2h and occasional shaking.	Ammonium chloride	Copper column + Copperised cadmium colunm	3-nitroaniline + NED	Spectrophotometric 535 nm	Ahmed et al. 1996
Nitrate and Nitrite (simultaneous)	Liver pâtés	Homogenised with hot water and followed by purification and filtration by addition of disodium tetraborato and Carrez reagent (potassium hexacyanoferrate and zinc acetate)	Ammonium chloride + sodium tetraborate + EDTA	Cadmium reductor column	Sulphanilamide NED Phosphoric acid	Spectrophotometric 530 nm	Pinho et al. 1998
Nitrite and nitrate	Vienna sausages	Chopped and placed with hot water in a flask that was boiled for 2h and cooled, then 2g of active carbon were added to the clean.		Photochemical reduction in a photoreactor (a low- pressure mercury lamp)	H ₂ SO ₄ /iodine	I ³⁻ /I ⁻ System potentiostat	Torró et al. 1998
Nitrate and Nitrite (simultaneous)	Beef	Sample was mixed with sand and homogenised in a mortar and digested in a bath (100°C) during 2h and shaking occasionally.	Ammonium chloride	Copperized cadmium reductor column	Potassium permanganate at 23°C	Spectrophotometric 530 nm	Ensafi and Kazemzadeh 1999
Nitrate and Nitrite (simultaneous)	Beef	Sample was mixed with sand and homogenised in a mortar and digested in a bath (100°C) during 2h and occasional shaking.	HCl +KCl	Two sequential column Copper and copper-coated cadmium	Safranine O	Spectrophotometric 520 nm	Kazemzadeh and Ensafi 2001
Nitrite	Meat products mortadella	Homogenised with water containing 4 drops of NaOH.	Detilled water		HCl nitrite zone O_2 gas carrier	Spectrophotometric 205 nm	Haghighi and Tavassoli 2002
Nitrate and Nitrite	Pork sausages and hot-dogs	Homogenised with hot water and followed by clarification of the extract by addition of disodium tetraborato and Carrez reagent	Ammonium chloride + EDTA	Cadmium cooper reductor column	Ferrous and thioacyanate solution	Spectrophotometric 460 nm	Andrade et al. 2003

		(potassium hexacyanoferrate and zinc acetate)					
Nitrate and Nitrite (simultaneous)	Cured meat	Homogenised with hot water and followed by purification and filtration by addition of disodium tetraborato and Carrez reagent (potassium hexacyanoferrate and zinc acetate)	Ammonium chloride + EDTA	Copperized cadmium mini column	Sulphanilamide NED HCl	Spectrophotometric 538 nm	Oliveira et al. 2004
Nitrite	Homogenised meat samples	Homogenised with hot water and transferred flask to a bath and left for 2h, occasional shaking.	H ₂ SO ₄	Gas diffusion membrane- based optical flow-trough sensor	Sulphanilamide and NED and Hydrochloric acid	Optical flow-trough sensor (absorbance or reflectante)	Frenzel et al. 2004
Nitrite	Meat sausage	Homogenised with hot water and transferred flask to a bath (80°C) and left for 2h, occasionally shaking.	Detilled water		Safranine O	Spectrophotometric 520 nm	Penteado et al. 2005
Nitrate and Nitrite	Frankfurter sausages	Homogenised with hot water and transferred flask to a bath (100°C) and left for 2h, occasional shaking.	Ammonium chloride+ EDTA	Cadmium reductor column	Sulphanilamide NED, Phosphoric acid	Spectrophotometric 540 nm	Ruiz-Capillas et al., 2006
Nitrite and Protein bound nitrite	Frankfurter sausages and Spanish dry sausages	Homogenised with hot water and transferred flask to a bath (100°C) and left for 2h, occasional shaking.	Buffer 7. and 7.5	Cadmium reductor column	Sulphanilamide NED, Phosphoric acid	Spectrophotometric 540 nm	Ruiz-Capillas et al. 2007
Safranine O (3,7	-diamino-2,8-dim	ethyl-5-phenylphenazinium chloride)					

Table III.- Sulphite determination by flow injection analysis in muscle based foods.

	Sample	Extracion	Hydrolysis	Donor Reagent	Separation	Acceptor Reagent (colorimetric reagent)	Detection system	Ref	
Sulphites	Gulf shrimp, shrimp and other food and beverages (wine, juice, guacamole, apricots, potatoes, onions, apples lettuce, cabbage)	Sodium Tetrachloromercurate (TCM)		Sulfuric acid	Gas diffusion cell	Malachite green	Spectrophotometric 615 nm	Sullivan et al. 1986	
Total sulphites	Shrimp and other food and beverages (patatoes, dried pineapple, white wine)	ТСМ	NaOH	Sulfuric acid	Gas diffusion cell	Malachite green	Spectrophotometric 615 nm	Sullivan et al. 1990	
Total Sulphite	Food and beverages	TCM	NaOH	Sulfuric acid	Gas diffusion cell	Malachite green	Spectrophotometric 615 nm	AOAC 2005 (n° 990.29)	
Sulphite 615 nm (n° 990.29)									