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Green Alternatives to Nitrates and Nitrites in Meat-Based Products – A Review

Dr Fatma Gassara^a, Miss Anne Patricia Kouassi^b, Dr Satinder Kaur Brar^c & Khaled Belkacemi^d
Professor

^a Institut national de la recherche scientifique Centre Eau, Terre & Environnement/Centre for Water, Earth and Environmen, Centre Eau, Terre & Environnement/Centre for Water, Earth and Environment, 490 de la Couronne, QUEBEC, Quebec, G1K 9A9 Canada, Email:

^b Université Laval, Département des sols et de génie agroalimentaire, Pavillon Paul-Comtois, 2425, rue de l'Agriculture, QUEBEC, Quebec, G1V 0A6 Canada, Email:

^c Institut national de la recherche scientifique Centre Eau, Terre & Environnement/Centre for Water, Earth and Environmen, Centre Eau, Terre & Environnement/Centre for Water, Earth and Environment, 490 de la Couronne, Québec (Qc), QUEBEC, Quebec, G1K 9A9 Canada,

^d Université Laval, Faculté des sciences de l'agriculture et de l'alimentation Sces agriculture et alimentation-, Dép. sols et génie agroalimentaire, 2425, rue de l'Agriculture, QUEBEC, Quebec, G1V 0A6 Canada, Email:

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Green Alternatives to Nitrates and Nitrites in Meat-Based Products – A Review

Dr Fatma Gassara

Email: fatma.gassara@ete.inrs.ca

Institut national de la recherche scientifique Centre Eau, Terre & Environnement/Centre for Water, Earth and Environmen, Centre Eau, Terre & Environnement/Centre for Water, Earth and Environment, 490 de la Couronne, QUEBEC, Quebec, G1K 9A9 Canada

Miss Anne Patricia Kouassi

Email: anne-patricia.kouassi.1@ulaval.ca

Université Laval, Département des sols et de génie agroalimentaire, Pavillon Paul-Comtois, 2425, rue de l'Agriculture, QUEBEC, Quebec, G1V 0A6 Canada

Dr Satinder Kaur Brar*

Email: satinder.brar@ete.inrs.ca

Affiliation 1:

Institut national de la recherche scientifique Centre Eau, Terre & Environnement/Centre for Water, Earth and Environmen, Centre Eau, Terre & Environnement/Centre for Water, Earth and Environment, 490 de la Couronne, Québec (Qc), QUEBEC, Quebec, G1K 9A9 Canada

Professor Khaled Belkacemi

Email: Khaled.Belkacemi@fsaa.ulaval.ca

Université Laval, Faculté des sciences de l'agriculture et de l'alimentation Scs
agriculture et alimentation-, Dép. sols et génie agroalimentaire, 2425, rue de
l'Agriculture, QUEBEC, Quebec, G1V 0A6 Canada

Introduction

Nitrates and nitrites are present everywhere in the environment, in water, soil, air, plants and food. They are used as fertilizers, as explosives and also as preservative agents in foods particularly against *Clostridium botulinum*. They are natural chemical substances which are obtained from the oxidation of nitrogen by the microorganisms. Oral reduction of nitrate is the most important source of nitrite, accounting for approximately 70-80% of the human total nitrite exposure. About 5-7% of all ingested nitrate is converted to nitrite at the base of the tongue, where nitrate-reducing bacteria are present (Chan, 2011). For subjects with a high rate of conversion, this figure may be up to 20% (Eisenbrand et al., 1980). Excessive use of nitrates and nitrites not only presents a health hazard but may also result in nitrite burn which is a green or white discoloration in the cured meat (Montana Meat Processors Convention, 2001). The toxic effects of nitrates are due to its endogenous conversion to nitrite. The Acceptable Daily Intake (ADI) for nitrate is 0-3.7 mg/kg bw/day (expressed as nitrate ion) (Thomson, 2004). It can lead to risks to human health and the environment. The health effect of most concern to the U.S. EPA for children is the blue baby syndrome (methemoglobinemia) (Fan, Willhite & Book, 1987). The blue baby syndrome leads to the blue coloration of the skin of babies due to high nitrate concentrations in their blood. The nitrate binds to hemoglobin (the compound which carries oxygen in blood to tissues in the body), and results in chemically-altered hemoglobin (methemoglobin) that impairs oxygen delivery to tissues, resulting in the blue color of the skin (USEPA, 2007).

Nitrates and nitrites have been associated, at high level, with increased incidence of cancer in adults, combining with secondary or tertiary amines to form N-nitroso derivatives, and possible increased incidence of brain tumors, leukemia and nasopharyngeal problems. The U.S. EPA concluded that there was conflicting evidence in the literature as to whether exposure to nitrate or nitrites was associated with cancer in adults and in children. The types of cancers studied included non-Hodgkin's lymphoma as well as stomach and gastric cancers in adults; and brain tumors, leukemia, and nasopharyngeal cancers in children. Their addition is however very limited (USEPA, 2006). Industries use nitrates and nitrites for the stabilization of the red color of meats (Honikel, 2008), inhibition of the development of toxic microorganisms, by decreasing the oxidation of lipids and to improve the flavor (Pegg and Shahidi, 2000). Nitrates and nitrites are preferred as they are less expensive for the properties that they offer. Meanwhile, the industries have not found a better and economical substitute to these nitrites and nitrates. Due to strong political and social will in many countries to establish stricter regulations, the industries are obliged to find greener substitutes to nitrates and nitrites.

Alternatives of nitrates and nitrites have been already investigated (Stoilova et al., 2007; Shirin and Prakash, 2010; Stoilova et al., 2006; Grohs and Kunz, 2000). Chemical agents, such as ascorbate and α -tocopherol, lactic-acid-producing organisms, potassium sorbate, or treatments, such as irradiation (National Academy of Sciences, 1982) that have been used as nitrite-nitrate substitutes. A number of studies have been also carried out to investigate the properties of aromatic herbs, fruits, essential oils and spices and like clove, ginger, pepper or garlic (Menon and Garg, 2001). Cinnamaldehyde, the major constituent of cinnamon (*Cinnamomum cassia*) has

been reported to possess antibacterial activity and antioxidant properties (Chang et al., 2001). An essential oil is a complex mixture of several volatile aroma compounds belonging to different classes of organic chemistry: phenols (eg carvacrol), hydrocarbon (terpene compounds such as limonene), alcohols (eg linalool), aldehydes (eg cinnamaldehyde), ketones (eg menthone). Most of these compounds have antimicrobial properties, but they are volatile compounds which contain the most important antimicrobial properties, particularly phenols, alcohols and aldehydes: carvacrol (oregano, savory), eugenol (sheet Ceylon cinnamon, clove), linalool (coriander), cinnamaldehyde (Chinese cinnamon) (Oussalah, 2007). There are more than 1340 plants with defined antimicrobial compounds, and over 30,000 components have been isolated from phenol group-containing plant-oil compounds and used in the food industry (Tajkarimi et al., 2010). Researchers have found a positive linear correlation between phenolic compounds, primarily phenolic acids and flavonoids, and the antioxidant capacity of herbs and spices (Zheng, 2001).

The purpose of this review is to present nitrates and nitrites, their role in the environment, presence in natural products, their role in preservation of meat and the reason why it is important to find alternatives. Selected alternatives, such as spices and natural products are also presented in this review due to their preservation properties and safety.

I-Definition of nitrates and nitrites

1. Nitrites and nitrates chemical composition

Nitrates and nitrites occur naturally as compounds consisting of nitrogen and oxygen, although in different chemical structures. The nitrogen cycle contains both compounds. The chemical difference between nitrate and nitrite lies in one additional oxygen atom. Nitrite is one part nitrogen and two parts oxygen. The nitrogen cycle comprises oxidation of nitrite, NO_2 into NO_3 , or nitrate.

2. Sources of nitrates and nitrites

2.1. Natural sources of nitrates and nitrites

2.1.1. Soil

Nitrate is a natural material in soils. Adequate supply of nitrate is necessary for good plant growth. More than 90 percent of the nitrogen is probably absorbed by plants in the nitrate form (Brown et al., 2007). Chemical nitrogen fertilizer is often in the ammonium nitrogen (NH_4^+) form and is rapidly converted to nitrate (NO_3^-) in the soil. The crop growth is essentially the same whether nitrogen fertilizer is applied as ammonia (NH_3), ammonium or nitrate (NO_3^-) (Brown et al., 2007). Chemical fertilizers may be composed of ammonium nitrate, ammonium phosphates, ammonium sulfate, various nitrate salts, urea and other organic forms of nitrogen. Soil organic matter contains about 5 % of N. For each 1 percent of organic matter, 7-inch plow layer of an acre (about 2,000,000 pounds of soil) contains about 1,000 pounds of N. Microorganisms must change organic nitrogen to ammonium or nitrate before plants can use it. Usual release of available N from soil organic matter is 1 to 4 percent annually, depending on soil texture and weather conditions (Brown et al., 2007). Nitrogen, present or added to the soil, is subject to several changes (transformations) that dictate the availability of N to plants and

influence the potential movement of NO_3^- to water supplies (O'Leary, 1994). Animal manure is an excellent source of nitrogen and can significantly contribute to soil improvement. Animal manure contains about 10 pounds of N per ton, poultry manure about 20 pounds; and legume residues 20 to 80 pounds. About half of this organic nitrogen may be converted to nitrate-nitrogen and become available for plant use the year it is added to the soil. However, it is low in phosphorus content. Excessive manure applications can result in toxic levels of nitrate in forage crops the same as excessive use of chemical nitrogen fertilizer (Agbede and Ojeniyi, 2009). Adding phosphate fertilizer to manure can reduce the nitrate content in the crop produced. Effluent from animal waste treatment facilities may lose about 50 percent of its nitrogen to the atmosphere as it is applied to soils. However, applications of large quantities of effluent or solid waste can add excessive amounts of nitrogen to the soil. Applying large amounts per acre repeatedly to the same area may add more nitrogen to the soil system than can be used. Using feed additives in livestock feeding may contribute significant concentrations of certain elements such as copper, zinc, arsenic or others to the solid animal waste collected in lagoons or similar facilities. Such wastes continuously applied to soils may eventually result in soil levels toxic to plants and possibly to animals that consume the crop.

2.1.2. The atmosphere

Nitrogen in our atmosphere is an inert molecule at ambient temperature. The nitrogen in the air is also essential for life on Earth. It is incorporated into amino acids and proteins, and is part of the nucleic acids, such as DNA and RNA (Encyclopedia of Earth, 2011). However, the nitrogen atom itself is one of the chemical elements which can change its state of oxidation widely. The outer shell of five electrons (s^2p^3) can take up three additional electrons giving the nitrogen an

oxidation status of N^{3-} as it exists in ammonia (NH_3) or amines or it can release five electrons forming N^{5+} as it exists in nitrate NO_3^- . Atmospheric nitrate concentrations ranging from 0.1 to 0.4 g/m^3 have been reported, the lowest concentrations being found in the South Pacific (Prospero and Savoie, 1989; World Health Organization 2004). High levels of aerosol nitrate measured at this northern Canadian location were about $0.40\text{--}0.55 \text{ }\mu\text{g/m}^3$ between the years 2000 and 2005 (Environment Canada, 2010). Higher concentrations ranging from 1 to 40 g/m^3 have also been reported, with annual means of $1\text{--}68 \text{ g/m}^3$. Mean monthly nitrate concentrations in air in the Netherlands range from 1 to 14 g/m^3 (Janssen et al., 1989; World Health Organization 2004). Indoor nitrate aerosol concentrations of $1.1\text{--}6.6 \text{ g/m}^3$ were found to be related to outdoor concentrations (Yocom, 1982; World Health Organization 2004).

2.1.3. Plant

Nitrates occur naturally in vegetables and plants. Nitrite and nitrates occur naturally in vegetable as a consequence of the nitrogen cycle whereby nitrogen is fixed by bacteria.

Beetroot, broccoli, cabbage, celery, lettuce, radish and spinach have been reported to contain high concentrations (greater than 1000 mg/kg) of nitrate. In contrast, nitrite concentration in fresh vegetables is generally low (less than 1 mg/kg and not above 20 mg/kg) (Meah, 1994, Petersen and Stoltze 1999, Chung et al, 2003). Thus, nitrates are naturally present in plants, often at concentrations higher than the allowed uptake.

2.1.4. Water

Nitrates occur naturally in wastewater and drinking water. Nitrate is used mainly in inorganic fertilizers. It is also used as an oxidizing agent and in the production of explosives, and purified

potassium nitrate is used for glass making. Sodium nitrite is used as a food preservative, especially in cured meats. Nitrate is sometimes also added to food to serve as a reservoir for nitrite. Nitrate can reach both surface water and groundwater as a consequence of agricultural activity (including excess application of inorganic nitrogenous fertilizers and manures), from wastewater treatment and from oxidation of nitrogenous waste products in human and animal excreta, including septic tanks (World Health Organization 2011). Nitrite can also be formed chemically in distribution pipes by *Nitrosomonas* bacteria during stagnation of nitrate-containing and oxygen-poor drinking-water in galvanized steel pipes or if chloramination is used to provide a residual disinfectant and the process is not sufficiently well controlled.

2.1.5. Endogenous biosynthesis of nitrate

Nitrosating agents (NAs; N_xO_y form) can react under certain conditions with nitrosatable compounds (NCs) to form *N*-nitrosamines and *N*-nitrosamides (hereafter nitrosamines and nitrosamides), collectively called *N*-nitroso compounds (NOCs): an equation showing the formation of *N*-Nitroso compounds ($NAs + NCs \rightarrow NOCs$). This reaction is called *N*-nitrosation or simply nitrosation (Health Canada, 2013). Human beings are exposed to various types of nitrosating agents through diet, drinking water and tobacco smoke. These substances can also be synthesized endogenously from ingested nitrate and nitrite (Bartsch et al., 1988; Brambilla and Martelli, 2005).

Gastrointestinal infections greatly increase nitrate excretion via endogenous (non-bacterial) nitrate synthesis, probably induced by activation of the mammalian reticulo endothelial system (FAO/WHO, 1996; Lundberg et al., 2009). This endogenous synthesis of nitrate, presented in Figure 1 complicates the risk assessment of nitrate. Increased endogenous synthesis of nitrate, as reported in animals with induced infections and inflammatory reactions, was also observed in humans. Infections and non-specific diarrhoea played a role in the increased endogenous synthesis of nitrate (Gangolli et al., 1994; World Health Organization 2011). These observations are all consistent with the induction of one or more nitric oxide synthases by inflammatory agents, analogous to the experiments described in animals and macrophages. In humans, saliva is the principal site for the formation of nitrite. About 5% of dietary nitrate is converted to nitrite (Gangolli et al., 1994; World Health Organization 2011). A direct correlation between gastric pH, bacterial colonization, and gastric nitrite concentration has been observed in healthy people with a range of pH values from 1 to 7 (Mueller et al., 1986, Viani et al., 2000; Moigradean et al., 2008).

2.2. Use of nitrates and nitrites as additives in food products

Nitrates and nitrites are used as food additives in processed food as preservatives and colour fixatives in meat, poultry, fish and cheese (European Commission, 1995). Sodium nitrite has been used extensively in curing meat and meat products, particularly pork products, such as ham, bacon and frankfurters; certain fish and poultry products are also cured with brines that contain sodium nitrite. The process may include dry curing, immersion curing, or direct addition or injection of the curing ingredients. Curing mixtures are typically composed of salt (sodium

chloride), sodium or potassium salts of nitrite and nitrate and seasonings. Sodium nitrite acts as a colour fixative and inhibits the growth of bacteria, including *Clostridium botulinum*, which is the source of botulism toxin. Nitrite is a relatively strong reducing agent that has antibacterial properties; however, the preservation of foodstuffs can be attributed to a large degree to the high concentration of salts (including nitrate) that are employed during the curing process. In addition, nitrate can act as a reservoir whereby nitrite may be formed by microbiological reduction (Pokorny *et al.*, 2006).

3. Physicochemical properties of Nitrites and Nitrates

3.1. Role of nitrates and nitrites as antioxidant

One of the most important properties of nitrite is its ability to effectively delay the development of oxidative rancidity. This prevention occurs even in the presence of salt, which is a strong oxidant. Lipid oxidation is considered to be a major reason for the deterioration of quality in meat and poultry products which often results in the development of rancidity and subsequent warmed over flavors (Vasavada and Cornforth, 2005). The oxidation of unsaturated fats occurs more quickly in uncured, cooked meats than in cured meats because iron that is not bound to nitric oxide can act as a catalyst for oxidation. The antioxidant effect of nitrite is likely due to the same mechanisms responsible for cured color development involving reactions with heme proteins and metal ions, chelating of free radicals by nitric oxide, and the formation of nitroso- and nitrosyl compounds having antioxidant properties (Sebranek, 2009). The antioxidant effect of nitrite has been well documented (Townsend and Olson, 1987; Pearson and Gillett, 1996; Pegg and Shahidi, 2000; Honikel, 2004). Nitrite has been shown to inhibit warmed over flavor

development at relatively low levels. The addition of nitrite to kavurma, a type of fried meat, could significantly reduce the level of oxidation, measured by thiobarbituric acid, peroxide, and free fatty acids, as compared to a control which did not have nitrite added (Yetim et al. (2006). Sato and Hegarty (1971) reported significant inhibition of warmed over flavor development at a 50 ppm nitrite level with complete inhibition at a 220 ppm level. Investigating the effect of nitrite on lipid oxidation in various muscle systems, Morrissey and Tichivangana (1985) reported as little as 20 ppm nitrite was sufficient to significantly ($P < 0.01$) inhibit oxidation of lipid in fish, chicken, pork, and beef systems. Despite the antioxidant power of nitrites and nitrates, industries have to find healthier and greener alternatives for these chemicals products which have drawbacks far astounding than the advantages.

3.2. Antibacterial activity of nitrites and nitrates

Nitrites and nitrates play a key role in cured meat as a bacteriostatic and bacteriocidal agent. Nitrite is strongly inhibitory to anaerobic bacteria, most importantly *Clostridium botulinum* (Sofos et al.,1979) and contributes to control of other microorganisms, such as *Listeria monocytogenes*. The effect of nitrite and the likely inhibitory mechanism differs in different bacterial species (Tompkin, 2005). The effectiveness of nitrite as an antibotulinal agent is dependent on several environmental factors including pH, sodium chloride concentration, reductants and iron content among others (Tompkin, 2005). The reaction sequences involving nitric oxide are probably an important part of the antimicrobial role of nitrite in cured meat. For example, some researchers have suggested that nitrous acid (HNO_2) and/or nitric oxide (NO) may be responsible for the inhibitory effects of nitrite (Tompkin, 2005). As nitrite reactivity is

key to microbial inhibition (one indicator of this is the strong dependence on pH), there are questions raised on the fact that whether ingoing or residual nitrite is most critical to antimicrobial effects.

Tompkin (2005) concluded that residual nitrite at the time of product temperature abuse is critical to antibotulinal effects and that depletion of residual nitrite during product storage will reach some point at which inhibitory effects are also depleted. Other compounds have the same antimicrobial properties as nitrites and nitrates; if the industries use these compounds, it is also for their action on color and meat taste .

3.3. Action of nitrites and nitrites on color and taste

The red colour of cured meat products is one of the important effects of nitrite in meat products. The red colour develops in a number of complicated reaction steps until NO-myoglobin (Fe^{2+}) is formed. Myoglobin exists in a muscle in three states, in which the cofactor haem, a porphyrin ring with an iron ion in its centre binds different ligands or in which the iron exists in the Fe^{2+} or Fe^{3+} state. In the native myoglobin, the porphyrin moiety is supported in the ligand binding by amino acids of the protein in the neighbourhood. In the $\div\text{original}\emptyset$ state, myoglobin with Fe^{2+} in the porphyrin cofactor does not bind any ligand by a water molecule. In the presence of oxygen, the myoglobin can bind an O_2 molecule and it becomes bright red. The iron ion is in the Fe^{2+} state. However, oxygen and other oxidizing agents, such as nitrite can oxidize the Fe^{2+} to Fe^{3+} . The formed metmyoglobin (MetMb) is brown. The $\div\text{original}\emptyset$ myoglobin (Mb), oximyoglobin (MbO_2) and the metmyoglobin occur together in meat. In a muscle in a live animal, there is very

little metmyoglobin which increases post-mortem with the disappearance of oxygen except when meat is MAP-packed with high oxygen content (Honikel, 2008).

Oxygen and NO are biatomic molecules. A similar biatomic molecule, CO also binds very tightly to myoglobin. In some countries (e.g. USA and Norway) modified atmosphere packaging (MAP) of meat with 162% CO is permitted. By reducing enzymes or chemical reactions with reducing agent, such as ascorbate, Fe^{3+} is reduced to Fe^{2+} . The NO formed from N_2O_3 can bind to the myoglobin (Fe^{2+}) and form a heat stable nitrosylmyoglobin (NO-myoglobin). Oximyoglobin is not heat stable and dissociates faster. The meat turns grey or brown. On heating the NO-myoglobin, the protein moiety is denatured but the red NO-porphyrin ring system (often called nitroso-myochromogen) still exists and is found in meat products heated to $120 \pm 1^\circ\text{C}$. This heat stable red colour will change on bacterial spoilage and it fades in Ultra Violet light. The transformation of myoglobin has been presented in details in Figure 2. The first one is advantageous as the consumer recognizes spoilage in fresh meat which also changes colour on spoilage. In most recent years, the riddle about the red colour of cured raw hams such as Parma ham without added nitrite or nitrate has been solved. Various authors proved that Fe^{2+} in the porphyrin ring was exchanged with Zn^{2+} which gives the products a pleasant red colour. Nitrite addition prevents the exchange of the cured meat colour (Adamsen, Moller, Laursen, Olsen, & Skibsted, 2006; Moller, Adamsen, & Skibsted, 2003; Parolari, Gabba, & Saccani, 2003; Wakamatsu, Nishimura, & Hattori, 2004; Wakamatsu, Okui, Ikeda, Nishimura, & Hattori, 2004).

Nitrite is also responsible for the production of characteristic cured meat flavor, though this is probably the least well understood aspect of nitrite chemistry (Pegg & Shahidi, 2000). It is easy to distinguish cooked, cured ham from fresh roast pork on the basis of flavor but the chemical identity of distinguishing flavor components in cured meat has eluded numerous researchers. Some of the flavor difference may be due to the suppression of lipid oxidation by nitrite but other antioxidants do not produce cured meat flavor. If nitrite does, in fact, form some volatile flavor factors, this would represent yet another reaction product of nitrite in cured meat.

4. Regulation of nitrite and nitrate

Meat products manufactured in the United States are heavily regulated by the United States Department of Agriculture (USDA) and Food Safety and Inspection Service (FSIS). The amount of ingoing sodium or potassium nitrite in comminuted products manufactured in the United States is 156 ppm. The amount of ingoing sodium or potassium nitrite in comminuted products manufactured in the United States is 156 ppm (Sebranek and Bacus, 2007). This is based on the green weight of the meat block (which is different in other countries). According to regulations, dry cured products are restricted to 625 ppm of ingoing sodium nitrite or potassium nitrite. Immersion cured and massaged or pumped products are limited to 200 ppm ingoing sodium nitrite or potassium nitrite. According to the USDA, a minimum of 120 ppm of ingoing sodium nitrite is required for all cured "Keep Refrigerated" products. The only instance in which the rule is not in effect is when "the establishment can demonstrate that safety is assured by some other preservation process, such as thermal processing, pH or moisture control" (Sebranek and Bacus,

2007). Neither nitrate nor nitrite is permitted in baby or toddler food. Nevertheless, the food is safe for consumption due to the sterilization processing to which all baby food is subjected. Health Canada has identified maximum nitrites/nitrate levels in the Food and Drug Regulation. These levels are maximum of 200 ppm in cured meat and meat by-products (except bacon) and maximum of 100 ppm in bacon. These levels are well above those needed to stop the growth *Clostridium botulinum* spores. A complete ban on the use of nitrates and nitrites in foods has not been implemented because of the beneficial uses as preservatives and particularly their prevention of *Clostridium botulinum* growth. There is also some scientific evidence suggesting that low levels of nitrates and nitrites (below 200ppm) pose no health concern (Health Canada, 2008).

Many countries have used other directives and regulations for the use of nitrites and nitrates in meat products. The European Union (EU) has also promulgated its regulation and directives 2006/ 52/ DEC (Directive 2006). The use of nitrites and nitrates was limited. In general, 150 mg nitrite/kg is allowed to be added to all meat products plus 150 mg/kg for unheated meat products. This is a maximum of 300 nitrites plus nitrates/kg. A large number of exceptions, such as dry cured bacon may have 425 mg of residual nitrites plus nitrates/kg. The toxicity of nitrates and nitrites depend on their concentration in meat. Due to regulations and the toxicity of nitrates and nitrites, industries must be more stringent in their standard (limit concentration addition) or find healthier alternatives for nitrates and nitrates.

5. Toxicokinetics of nitrates and nitrites

5.1. Absorption

Nitrates and nitrites are present in the environment under ionic form, and are non-volatile. There are two major sources of nitrate and nitrite in the human system: endogenous l-arginine \rightarrow NO synthase pathway and the diet. The main pathway of absorption of these substances is the ingestion. Dietary nitrate intake is considerable and nitrate is found as a naturally occurring compound in foods, such as vegetables, spinach and lettuce often containing up to 2500 mg.kg⁻¹ (World Health Organization 2011), fruit, cereals, fish, milk, and water also contain nitrate as a consequence of agricultural practices, such as the use of nitrogen-containing fertilizers and from animal waste (Dennis and Wilson, 2003). Low levels are generally found from these sources, except in the case of some vegetables. Nitrates and nitrites are also permitted as food additives in some foods, primarily as protection against botulism.

5.2. Distribution

Concerning their distribution, nitrate is reduced to nitrite, in the oral cavity, by the action of commensal bacteria found on the back of the tongue. The swallowed nitrite, under the influence of the acidic conditions in the stomach, becomes protonated to nitrous acid ($\text{NO}_2^- + \text{H}^+ \rightarrow \text{HNO}_2$). Once nitrite is formed, there are numerous pathways in the body for its further reduction to NO, involving haemoglobin, myoglobin, xanthine oxidoreductase ascorbate, polyphenols and protons (Lundberg et al., 2008). After the ingestion of nitrate, either in its dietary or medicinal form, there is a sharp rise in the salivary, plasma and urinary levels of nitrates and nitrites. In the plasma, the levels of nitrate rise within 30 min and peak at 3 h and are sustained for up to 24 h; In contrast, the levels of nitrites rise more gradually to a significant

level by 161.5 h and a plateau is reached at ~2.5 h and remains significantly elevated for up to 6 h (Sami et al., 2012).

About 5% of the dietary nitrate is reduced to nitrites in the saliva and the gastrointestinal tract. This value can reach 20% for individuals with a high rate of conversion (Thomson et al., 2007).

Methemoglobinemia is another health hazard attributed to nitrites, a condition where reduced iron (Fe^{2+}) in haemoglobin is oxidized by nitrite to Fe^{3+} , thus reducing the total oxygen-carrying capacity of the blood (Santamaria, 2006).

Nitrites are able to be produced endogenously. Nitrites may also combine with secondary or tertiary amines to form N-nitroso derivatives. Certain N-nitroso compounds have been shown to produce cancers in a wide range of laboratory animals (Codex, 1998).

5.3. Elimination

The elimination of nitrites is mainly carried out by urinary expression and maximal urinary nitrite excretion occurred approximately 4 to 6 h after consumption of each high-nitrate meal, with basal levels being reached by 24 h. Approximately 75% of the total ingested nitrate was excreted via urine. The other major routes of nitrate excretion have been reported to be saliva and sweat, for example, nitric oxide can be generated on the skin surface by the reduction of sweat nitrate (Pannala et al., 2003). Almost all the nitrates consumed are excreted in the urine within 24 h. Nitrites and nitrates are excreted in the kidneys. The nitrate is excreted in the urine

as such or after conversion to urea (Ellis et al., 1998). The details of the toxico-kinetics of nitrites and nitrates are provided in Figure 3.

6. Effect of nitrates and nitrites

6.1. Human health

Nitrates and nitrites have different effects on health. With relation to human system, the main toxic effects of nitrates are due to its endogenous conversion to nitrites (Thompson, 2004); Nitrites, on reacting with the iron of the hemoglobin of red blood cells, reduces the transport capacities of the oxygen by the haemoglobin of the blood. It, thus leads to the formation of methaemoglobinaemia or the blue baby syndrome.

The methaemoglobinaemia caused by nitrates in the drinking water is mainly observed when the child's age is less than 3 months. Bacterial contamination of water, gastrointestinal infections and inflammation with ensuing production of nitric oxide are major factors that may contribute to methemoglobinemia (Fan, 2011). The blue baby syndrome is named for the blue coloration of the skin of babies who have high nitrate concentrations in their blood. The nitrate binds to hemoglobin (the compound which carries oxygen in blood to tissues in the body), and results in chemically-altered hemoglobin (methemoglobin) that impairs oxygen delivery to tissues, resulting in the blue color of the skin. The blue coloration can be seen in the lips, nose, and ears in the early stages of blue baby syndrome, and extend to peripheral tissues in more severe cases (EPA, 2006).

Reduced oxygenation of the tissues can have numerous adverse implications for the child, such as coma and death. Toxic doses of nitrites responsible for methemoglobinemia range from 0.4 to more than 200 mg kg⁻¹ of body weight. The guideline for nitrite ion in infants is 3 ppm (US EPA, 2006). Exposure to higher levels of nitrates or nitrites has been associated with increased incidence of cancer in adults, and possible increased incidence of brain tumors, leukemia, and nasopharyngeal (nose and throat) tumors in children in some but not others. U.S. EPA concluded that there was conflicting evidence in the literature as to whether exposures to nitrate or nitrites are associated with cancer in adults and in children.

6.2. Effects on animal health

The effects of nitrates on animal health is due to livestock feeding which contains nitrites and nitrates. The nitrate is transformed into nitrite and in other N-nitroso compounds in the saliva of most monogastrics or in ruminants' rumen because of the microbial activity. The unfavorable effects on the health of the livestock result from an acute exposure to the nitrites due to the formation of methemoglobin in the blood. This can lead to the cyanosis and death at very high levels.

Clinical signs of acute nitrite toxicity in a range of livestock associated with methemoglobin are generally dose-dependent due to oxygen starvation and may include accelerated pulse, dyspnoea, muscle tremors, weakness, vomiting, unstable gait, and cyanosis leading to death. Symptoms of sub-chronic and chronic toxicity include reduction in feed intake, milk production in dairy

animals, rough hair and reduced weight gain or actual loss. In pregnant animals, low fertility and abortion, correlated with foetal hypoxia due to methemoglobine can also occur (Cockburn et al., 2010).

6.3. Effects on environment

Nitrites and nitrates have many harmful effects on the environment. Aquatic life is more vulnerable to nitrite intoxication, as nitrite, in the ambient water, can be taken up via gill epithelium and accumulated to very high concentrations in the body fluids (Jensen, 2003). The cycle of nitrate in water is presented in Figure 4.

However, more indications have shown that nitrates and nitrites could have fatal effects on the development of the aquatic organism in the first stages of life by limiting the capacity of the blood to transport the oxygen or by disrupting the acid-basic balance. Although nitrate in the natural concentrations generally has no mortal effect on the body it can cause growth delays or limited survival.

In the environment, nitrogen is found under its diatomic shape N_2 (inert gas). This form, unusable by plants and animals, is going to settle with other molecules as the hydrogen. It is consumed by blue-green algae - cyanobacteria - which live in surface water. Later, the nitrification produces nitrates and nitrites. Finally, denitrification, a reduction of nitrate, which returns the nitrogen to the atmosphere in its form N_2 , with CO_2 and nitrogen oxide, N_2O as

secondary product , greenhouse gas which contributes to destruction of the ozone layer in the stratosphere. The nitrogen cycle is presented in details in Figure 5.

Nitrates and nitrites are naturally occurring ions and are ubiquitous in the environment. Nitrate contamination of surface- and groundwater is a pervasive, worldwide problem, while nitrite is an important indicator of fecal pollution in water. According to the National Primary Drinking Water Regulations, nitrates are highly mobile in soil and have a higher potential to migrate to ground water due to higher solubility in water and weak retention by soil. Nitrates and nitrites do not volatilize and therefore are likely to remain in water until consumed by plants or other organisms. Ammonium nitrate is taken up by bacteria, and nitrate degradation is fast under anaerobic conditions. Nitrite is easily oxidized to nitrate, and nitrate is the more predominant compound of the two forms detected in groundwater (EPA, 2006). Nitrates are essential for the growth of vegetables, and are present in the composition of fertilizers natural as manure. Fertilizers and badly used pesticides pollute subterranean waters (by infiltration into the ground by rainwater and watering) and surface (streaming). The excessive use of fertilizers appreciably increases the quantity of nitrate in rivers and low depth ground waters.

The nitrate is nevertheless a beneficial natural element integrated into the nitrogen cycle and indispensable for the growth of vegetables. An excessive use of nitrates destabilizes this process: the rainwater, after infiltration, entails the nitrate fertilizers, which plants and grounds were not able to absorb, and then pollutes the fresh water.

Both nutrients, particularly in dissolved species, such as nitrate, nitrite, ammonium and phosphate, are easily assimilated by phytoplankton for growth and act as significant factors in the regulation of primary productivity in water bodies. Nitrogen concentrations in river and ground water increase as a response to increased agricultural activity and point-source discharges and were one of the most implicated species in eutrophication of water (Xinhai, 2010).

II- Alternative of nitrites and nitrates used for preserving meat products

1. Chemical Alternatives

Several studies have shown the presence of chemicals, such as sulfur dioxide, ethylene diamine tetraacetic acid, butylated hydroxyanisole BHA, fumarate esters, sodium hypophosphite, among others capable of inhibiting pathogens and/or lipid oxidation. The use of chemicals can be effective, but potential adverse effects of these products may appear over time. Prior to the 80s, it was not known that the use of nitrates/nitrites as preservatives can have deleterious effects on the health of consumers. Thus, the use of natural products is always preferable to the use of chemicals.

2. Vitamin

Several studies have shown that vitamins, such as α -tocopherol could be an effective alternative to nitrites and nitrates in meat products. In addition, it has been shown that the α -tocopherol can

inhibit the growth of pathogens. However, vitamins are often expensive which will make the meat process more expensive (Bhat et al., 2013).

3. Natural sources of nitrites and nitrates

It has been shown in the literature that there are certain products that naturally contain nitrites such as celery, lettuce, spinach, radishes, among others. Some researchers have used extracts of these natural products as substitutes of nitrite in meat products. However, nitrites/nitrates, coming from natural sources or not, present a potential harmful effect as chemical nitrites and nitrates, because even if its origin is from natural source, these nitrites will be transformed to nitrosamines in the human system leading to negative effects.

4. Agents preventing the formation of nitrosamines

There are products that inhibit the formation of nitrosamines and nitrosamides from nitrite and nitrate, such as ascorbate and ascorbic acid. These products can be used in the presence of nitrites in meat products to inhibit their transformation into nitrosamines and nitrosamides. The sequence of reactions of ascorbate preventing nitrosamine formation has not been fully elucidated. It may be due to the reduction of residual nitrite in meat products by ascorbate (EFSA, 2003) or the binding of NO to ascorbate and its retarded release. In the last decades, ascorbic acid and isoascorbate (erythorbate) has been used in cured meat batters. There is a

reaction of ascorbate with oxygen forming dehydroascorbate and thus reducing the amount of nitrite which could be oxidized to nitrate (Bertelsen, and Quist, 1994). However, ascorbate also seems to react with nitrite (nitrous acid or NO). Dahl, Loewe, and Bunton (1960), Fox and Ackerman (1968) and Izumi et al. (1989) showed that ascorbate also reacts with nitrite and binds the resulting NO. The bound NO seemed to react as NO with other meat ingredients. Ascorbate is also added to reduce the formation of nitrosamines. The sequence of reactions of ascorbate preventing nitrosamine formation has not been fully elucidated. It may be due to the reduction of residual nitrite in meat products by ascorbate (EFSA, 2003) or the binding of NO to ascorbate and its retarded release together with nitrite and salt. Nevertheless, it becomes clear that nitrite is a very reactive substance which undergoes many reactions in meat products and thus its use has to be controlled. In contrast, nitrites in meat products remain and pass through the stomach of the consumer where they can transform under the action of acidic pH of the stomach to nitrosated products. Similarly, nitrites themselves, without any transformation are harmful to consumers.

5. Spices and fruits

Spices are considered as good alternatives of nitrites due to their well known antibacterial and antioxidant properties. Rosemary, mace, oregano and sage have antioxidant properties that can delay the onset of rancidity in fats (Kim et al., 2011). Black pepper, white pepper, garlic, mustard, nutmeg, allspice, ginger, cinnamon and red pepper are known to stimulate the

Lactobacillus bacteria producing lactic acid, which increases the shelf life of meat products (Sallam et al. 2004). In this regard, the use of spices and their volatile compounds as natural preservatives in food can be an alternative to the use of chemical additives (Kim et al., 2011). The use of garlic powder may be very important as garlic is known for its antioxidant and antimicrobial properties (Kim et al., 2011). In addition, the use of garlic reduces the amount needed to ensure proper conservation that will not alter the taste of the meat products. It has also been shown that cloves and garlic in combination will inhibit all pathogenic bacteria (Yadav and Singh, 2004). The conjunction, physical-chemical properties and antimicrobial properties of garlic and cloves play an important role in the use of these spices as natural alternatives of nitrites and nitrates in food. However, these spices, if used in large quantities, they will damage the taste of food. Hence, the quantity of these spices has to be minimized to maintain the organoleptic and preservative properties of food.

5.1. Advantages in using spices as an alternative of nitrates and nitrites

Spices and herbs have been used since ancient times for different purposes. Originally, most of them were used to preserve food. Within this group, there are spices, cinnamon, cloves and turmeric. They possess bactericidal and fungicidal properties that can kill or inhibit the growth of organisms which could spoil the food. Pepper and cinnamon are reputed to be the best food preservatives.

Closely related to this function is the fact that the spices can disguise the bad taste or smell of foods. This is evident especially in warm places, where the heat promotes decomposition and is a leading cause of odors. Food processing technologies, such as chemical preservatives cannot eliminate food pathogens, such as *Listeria monocytogenes* or delay totally the microbial spoilage (Gutierrez et al., 2009).

These constituents form the characteristic of the spice, and possess medicinal and pharmacological properties with a possible impact on human health. India is known as the home of spices, and is also a leading producer of major spices (Wealth of India, 2001). Among the spice-growing countries, such as India, Sri Lanka, Indonesia, and Malaysia, these are used extensively as natural food flavorings. In addition to flavoring, the spices help in protecting food from oxidative deterioration, thereby increasing shelf-life, and also play a role in the body's defenses against cardiovascular diseases, certain cancers, and conditions, such as arthritis and asthma.

In fact, a part of the oxygen used by our body can produce free radicals. These become fatal for a certain number of organic molecules as proteins, lipids and DNA of cells. The free radicals are atoms which possess a free electron which can engender chemical reactions found in the process of cellular oxidation. This oxidation can lead to the development of diseases, such as cancer, cardiovascular diseases and diabetes. Antioxidants, present in these natural plants, act to protect cells of degradation, reacting with free radicals to make them harmless. Their properties are mainly derived from the phenols or thiols present in these products

The first scientific studies of the preservation potential of spices, describing antimicrobial activity of cinnamon oil against spores of anthrax bacilli were reported in the 1880s. Moreover, clove was used as a preservative to disguise spoilage in meat, syrups, sauces and sweetmeats. In the 1910s, cinnamon and mustard were shown to be effective in preserving applesauce. Since then, other spices, such as allspice, bay leaf, caraway, coriander, cumin, oregano, rosemary, sage and thyme, have been reported to have significant bacteriostatic properties (Burt, 2004; Ceylan and Fung, 2004; Gutierrez et al., 2008a; Jayaprakasha et al., 2007; Tajkarimi et al., 2010).

These condiments can serve as disinfecting agents in the case of ground pepper and clove, treatment of digestive disorders for cinnamon, mustard, some cumin and saffron, anti-inflammatory drug as in the case of cumin, aphrodisiac in the case of the ginger and the hot pepper.

Natural antimicrobials have been identified in herbs and spices and several studies have been reported on the preservative action of spices or their essential oils. Among these natural antimicrobials are eugenol from cloves, thymol from thyme and oregano, carvacrol from oregano, vanillin from vanilla, allicin from garlic, cinnamic aldehyde from cinnamon, and allyl isothiocyanate from mustard (as reviewed by López-Malo et al., 2006).

5.2. Different types of spices

5.2.1. Cinnamon (*Cinnamomum zeylanicum*)

5.2.1.1. Characteristic of Cinnamon

This spice is constituted by the internal bark of four main species of cinnamon tree (Cinnamomum, Lauracée). *C. verum* allows production of a cinnamon of very good quality "Ceylon cinnamon". Other species lead to secondary lower-quality cinnamons called "cassias". *Cinnamomum zeylanicum* (L.), commonly known as cinnamon is rich in cinnamaldehyde as well as beta-caryophyllene, linalool and other terpenes. Cinnamaldehyde is the major constituent of cinnamon leaf oil and provides the distinctive odor and flavor associated with cinnamon. It is used worldwide as a food additive and flavoring agent, and the Food and Drug Administration lists it as "Generally Recognized as Safe-GRAS". (Nikos G. Tzortzakis, 2008).

5.2.1.2. Role of cinnamon in preservation

This spice was found to exert antioxidant activity in the fermented meat sausage (Al-Jalay et al., 1987), it possesses antibacterial properties against a large variety of microorganisms. Some of them are pathogenic (Morozumi, 1978; Huhtanen 1980; Hitokoto et al., 1980; Deans and Ritchie, 1987), inhibit growth and aflatoxin production of molds (Bullerman et al., 1977), inhibit food spoilage by yeast (Conner and Beauchat, 1984) and delay acid production by the starter bacterium, *Lactobacillus plantarum* (Zaika and Kissinger, 1979).

The antimicrobial and antifungal properties of cinnamon have also drawn great attention from many researchers (Delespaul et al., 2000; Chang et al., 2001 and Kim et al., 2004). Cinnamon possesses notable anti-allergenic, anti-inflammatory, anti-ulcerogenic, anti-pyretic, and anaesthetic activities (Kurokawa et al., 1998; Qin et al., 2009). Some evidence suggests that

cinnamon may be effective in the supportive treatment of cancer (Ka et al., 2003), infectious diseases, and complaints associated with modern lifestyle due to its antioxidant (Lin et al., 2003; Okawa et al., 2001; Toda, 2003), anti-microbial (Inouye et al., 2001; Smith-Palmer et al., 1998), and anti-inflammatory effects.

For preservation of food, cinnamon is known as an aperitif and digestion stimulant. Cinnamaldehyde has been reported to possess antibacterial activity against a wide range of bacteria (Chang et al., 2001), antioxidant properties (Gurdip et al., 2007).

A recent study investigated the effect of dietary supplementation with cinnamon and garlic powder as growth promoter agents on performance, carcass traits, immune responses, serum biochemistry, haematological parameters and thigh meat sensory evaluation in broilers (Toghyani et al., 2010). The study concluded that cinnamon as antimicrobial substances may inhibit intestinal pathogenic organisms and improve digestion and absorption; particularly inclusion of 2 g/kg cinnamon proved satisfactory results on performance indices and may have the potential to be applied as an alternative for antibiotics growth promoter in broiler's diet.

In another study (Tabak et al., 1999), dealing with the inhibitory effect on the growth and urease activity of extracts from stem bark of *Cinnamomum cassia* on *Helicobacter pylori*, it was shown that cinnamaldehyde at 200 mg/disk had the strongest inhibitory effect (90 mm) followed by eugenol 200 mg/disk (68 mm) and carvacrole 2000 mg/disk (66 mm). Cinnamaldehyde seems to be the main inhibitory component of cinnamon and the utilization of cinnamon extract to inhibit both growth and urease activity of *H. pylori* in vitro has proved to be more effective than the

actual thyme extract. The efficiency of cinnamon extracts in liquid medium and its resistance to lower pH levels may enhance its effect in the human stomach.

5.2.2. Clove (*Eugenia caryophyllus*)

5.2.2.1. Characteristic of cloves

The dried flower buds (*Caryophylli floss*) are used for medicinal and culinary purposes and an essential oil is also distilled. The taste is hot, intense, fresh, with a peppery taste and a touch of oriental fragrance. They are not soluble in water but can be dissolved in alcohol.

Cloves control nausea and vomiting, improve digestion, protect against internal parasites, cause uterine contractions and are strongly antiseptic. The major flavor component is eugenol.

5.2.2.2. Role of cloves in preservation

The eugenol inhibits prostaglandin formation, which explains the anti-inflammatory and analgesic effect, but the herb has further antiseptic, antispasmodic and carminative properties. Among the many properties, it is antiplatelet, antiviral, fragrant and flavoring properties, with a bacterial inhibition of 75-100% (Tajkarimi, 2010).

During conservation, essential oils are well known inhibitors of microorganisms (Burt, 2004). Clove oil showed its antimicrobial activity in a study based on the inhibitory effect of clove oil on *Listeria monocytogenes* in meat and cheese (Menon and Garg, 2001). Clove oil managed to limit the proliferation of *Listeria monocytogenes* with 0.5% and 1 %, 30°C and 7°C. Soliman & Badeaa (2002) found that 500 ppm of cinnamon oil can inhibit *A. flavus*, *A. parasiticus*, *A. ochraceus* and *Fusarium moniliforme* on PDA. August (1978) reported that higher concentrations of cinnamon oil and clove oil could also inhibit the asexual spores of fungi. Mixtures of cinnamon and clove oils are therefore an interesting alternative to use other chemical preservatives and appear well suited to use in active packaging systems (Matan, 2006).

Many studies have shown the efficiency of clove associated with other spices, such as cinnamon. The efficiency of cinnamon oil and clove oil, as an antibacterial agent, was reported by Ouattara et al. (1997) and Pradsad and Seenayya (2000). The main inhibitory components of cinnamon oil and clove oil are believed to be cinnamaldehyde and eugenol, respectively (Jayatilaka et al., 1995; Porta et al., 1998), and their effectiveness against molds and yeasts has been reported by López-Malo et al. (2002).

5.2.3. Cumin (*Cuminum cyminum* L.)

5.2.3.1. Characteristic of Cumin

Sometimes spelled cumin (*Cuminum cyminum* L.) is a flowering annual plant of the Umbelliferae family. This plant, which is one of the most important spices in the world, is native to India, Iran, the Mediterranean, and Egypt. There are two varieties of cumin: the white one

(more common) and the black one. The cumin is a biennial plant, which grows in light soils. It is sown in autumn or at the beginning of the year. The seeds of cumin can contain 3 to 7 % of essential oil. Cumin, the aromatic seed spice, finds wide applications in foods, beverages, and traditional medicine, and is known to possess bioactive properties.

The cumin, pricked, spiced, flavor of hazelnut with a strong aromatic odor, has anti-spasmodic virtues and helps to digest the heavy food. Its aroma matches with the meat, the autumn vegetables, the salad, the cheese or the diverse pastries. It is a carminative, an astringent, a stomachic, and is useful in dyspepsia and diarrhea.

5.2.3.2. Role of Cumin in preservation

Studies have illustrated its application as a preservative in foods, as well as its antibacterial, antioxidant, hypoglycemic, hypolipidemic activities, in addition to its use in perfumery and flavoring liquors. Cumin is traded as whole product, in the ground form, or as an essential oil (Kanagal Sahana, 2011). Cuminaldehyde was found to be the main component at concentrations of 53.6% for seed oil and 40.5% for herb oil (El-Sawi et al., 2002).

Takahashi, Muraki and Yoshida (1881) reported that cumin oil contained mint sulfide as a trace constituent. Twelve years later, (Anon, 1993) and (Shaath and Azzo, 1993) reported that the main constituents of Egyptian cumin seed oil were cumin aldehyde, α -pinene, α -terpinene, α -mentha-1,3-dien-7-al, α -mentha-1,4-dien-7-al and *p*-cymene. Black cumin seeds have many biological properties including anti-tumor (El-Daly, 1998), anti-diabetic (Al-Hader et al., 1993), diuretic (Zaoui et al., 2000) and anti-bacterial (Kamali et al., 1998) activity.

5.2.4. Black pepper (*P. nigrum*)

5.2.4.1. Characteristic of black pepper

Berry stemming from a plant native of India, called pepper plant, pepper is a part of the family of Piperaceae and appears under the shape of clusters. Black pepper (*P. nigrum*) is a perennial plant and derives its name Piper, perhaps, from the Greek name for black pepper, Piperi (Rosengarten, 1973) and most of the European names for black pepper were derived from the ancient Indian language, Sanskrit, such as Pippali, the name for long pepper (*P. longum*). It was the great botanist Linnaeus (1753) who established the genus *Piper* in his *Species Plantarum*.

The essential oil in the berry contributes to the aroma, while the alkaloid piperine imparts the unique pungency. Oleoresin is extracted from the dry powdered berries by solvents, and is the product that imparts the unique aroma, flavor and pungency in pepper (Lewis et al, 1976) attributed blackening of pepper berries due to enzymatic oxidation of polyphenolic substrates present in the skin of green pepper.

Black pepper is valued mostly for its spicy aroma and piquant pungent taste. Oleoresin, produced by solvent extraction of dried powdered pepper, contains both aroma and pungency principles (Premi, 2000). The active constituent of pepper, piperine is sensitive to light and oxygen. Different products from black pepper available are ground pepper, pepper oil and oleoresin (Ravindran & Johny, 2001).

Pepper is associated with a number of functional properties, such as analgesic and antipyretic properties, antioxidant effects and antimicrobial properties (Kapoor et al., 1993). Piperine, an active ingredient in pepper, exerts substantial analgesic and antipyretic effects (Lee et al., 1984).

5.2.4.2. Role of black pepper in preservation

Cinnamon seems to have a lot of good properties for preservation in food. Nevertheless, studies on the organoleptic properties are still required; the cinnamon having a pronounced taste and a rather present smell. It would be necessary to measure in that case the proportion of this spice in the formulations without changing the properties looked for the preservation of meats. The organoleptic analyses are also appropriate with cloves. Cloves, on its own, have good anti-inflammatory, analgesic and antimicrobial properties. It can be associated with cinnamon, as mentioned above, and maybe with other spices, due to antioxidant properties, to improve this property.

It is obvious that organoleptic analyses are important for all the formulations of spices; in the case of black pepper, it has a spicy and strong aroma. Finally, it will be interesting to see if the cumin powder has the same properties than cumin oil given that most of articles deal only with cumin oil. Table 1 presents the summary of various properties of spices which help to understand their eventual applications.

5.2.5. Grapes

The grapes are good alternatives of nitrites and nitrates due to their important antioxidant properties and presence of higher concentration of phenolic compounds (Jayaprakasha et al., 2003), such as gallic acid, chlorogenic and caffeic acids, catechin, etc. According to studies

conducted at Oregon State University, raisins showed conservation properties similar to those of sodium nitrite in meat products and sausages. The combination of antioxidants, sugars and acids found in grapes proved to be as effective as sodium nitrite in maintaining food security. The use of grapes to replace sodium nitrite in meat has many health benefits. Firstly, while nitrite can form carcinogenic nitrosamines, grapes do not form them. Secondly, unlike sodium nitrite, the addition of grape is not accompanied by the addition of sodium. This is important for those who are dieting sodium hypo knowing that the addition of sodium nitrite concentration can cause problems with hypertension. Thirdly, raisins can improve the overall nutritional profile of meat products, because they are rich in antioxidants (Perumall et al., 2011) and help to maintain the taste of products in addition to being low fat. In addition, the grape is a fruit that is produced in large quantities and low cost. Its use as an additive for preservation of meat products could be interesting especially if it is added in the dry and ground form, which should facilitate its use and also reduce the amount used to maintain the organoleptic properties of meat products.

Conclusions

Curing of meat is a process known since ancient times with the intention to prolong the shelf-life of meat. The curing agents, nitrite and nitrate, react due to easily varying oxidation status of nitrogen into many derivatives with meat ingredients (Honikel, 2007). Nitrites and nitrates have antioxidant, anti microbial properties, preserve the red color of meat and are very cheap.

Nitrates have been measured in foods, and have been detected in vegetables, in preserved meats and baby foods. Due to stricter regulations, industries have to find a healthier alternative for

these products. When ingested, they can be dangerous in high dose and at long term, they can also be toxic to humans, animals and environment.

Among the various alternatives, the addition of spices is a very promising one; this alternative seems healthier and could be very interesting given the numerous properties of spices: antioxidants, antibacterial, among others. In fact, spices and herbs, which were originally added for improving taste, can also naturally and safely improve shelf life of food products. However, studies showed that the use of essential oil is more effective than the powder but nevertheless it was more expensive. Numerous spices, others than presented in this review, are also being studied, such as ginger, red pepper, coriander and many others. However, additional analyses are necessary, such as the organoleptic, chemical and microbiological analyses, to prove the reliability of these alternatives.

List of abbreviations

Acceptable Daily Intake (ADI)

U.S. EPA: U.S. Environmental Protection Agency

g/m³: microgram per cubic meter

mg/kg: milligram per kilogram

NAs: Nitrosating agents

NCs: nitrosatable compounds

NOCs: N-nitroso compounds

ppm: part per million

MetMb: metmyoglobin

Mb: myoglobin

MbO₂: oximyoglobin

MAP: modified atmosphere packaging of meat

NO-myoglobin: nitrosylmyoglobin

EU: European Union

BHA: butylated hydroxyanisole

PDA: Potato Dextrose Agar

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References

Agbebe, T.M., and Ojeniyi, S.O. (2009). Tillage and poultry manure effects on soil fertility and sorghum yield in southwestern Nigeria. *Soil and tillage research*. **104** : 74-81.

Anon, A. (1993). Analytical methods of committee. Application of gas-liquid chromatography to analysis of essential oils Part XVI. Monography for five essential oils. *Analyst*. **118** : 1089-1098.

Bartsch, H., Ohshima, H., Pignatelli, B. (1988). Inhibitors of endogenous nitrosation mechanisms and implications in human cancer prevention. *Mutat. Res-Fund Mol. M.* **202** : 307-324.

Bhat, R., Alias, A. K., and Paliyath, G. 2012. Progress in food preservation. Oxford, UK ; Ames, Iowa : Wiley, 2012.

Brambilla, G., and Martelli, A. (2005). Keynote comment: nitrosatable drugs, cancer, and guidelines for genotoxicity. *Lancet Oncol.* **6** : 538-539.

Brown, J.R., Marshall, C., and Smith, J. S. (2007). Nitrate in Soils and Plants. MU Extension G9804

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods—a review. *Int. J. Food Microbiol.* **94** : 223-253.

Ceylan, E., and Fung, D. Y. C. (2004). Antimicrobial activity of spices. *J. Rapid Methods and Autom. Microbiol.* **12** : 1655.

Chan, T. Y. K. (2011). Vegetable-borne nitrate and nitrite and the risk of methaemoglobinaemia. *Toxico. Lett.* **200** : 1076108.

Chang, S. T.; Chen, P. F., and Chang, S. C. (2001). Antibacterial activity of leaf essential oils and their constituents from *Cinnamomum osmophloeum*. *J. Ethnopharmacol.* **77** : 1236127.

Chung, S. Y., Kim, J. S., Hong, M. K., Lee, J. O., Kim, C. M., and Song, I. S. (2003). Survey of nitrate and nitrite contents of vegetables grown in Korea. *Food Add. Contam.* **20** : 621-628.

Cockburn, A., Brambilla, G., Fernández, M. L., Arcella, D., Bordajandi, L. R., Cottrill, B., Van Peteghem, C., and Dorne, J. L. (2010). Nitrite in feed: From Animal health to human health. *Toxicol. Appl. Pharmacol.*

Conner, D. E., and Beuchat, L. R. (1984). Effect of essential oils from plants on growth of spoilage yeasts. *J. Food. Sci.* **49** : 429-434.

Dennis, M.J., and Wilson, L.A. (2003). Nitrates and Nitrites. **In** : Encyclopedia of Food Sciences and Nutrition (Second Edition), pp. 413664141. Benjamin Caballero, York.

DIRECTIVE 2006/52/CE DU PARLEMENT EUROPÉEN ET DU CONSEIL du 5 juillet 2006 modifiant la directive 95/2/CE concernant les additifs alimentaires autres que les colorants et les édulcorants et la directive 94/35/CE concernant les édulcorants destinés à être employés dans les denrées alimentaires.

El-Sawi, S.A., and Mohamed, M.A. (2002). , Cumin herb as a new source of essential oils and its response to foliar spray with some micro-elements. *Food Chem.* **77** : 75680.

Ellis, A., Li, C. G., and Rand, M. J. (1998). Effect of xanthine oxidase inhibition on endothelium- dependent and nitrenergic relaxations. *Eur. J. Pharmacol.* **356** : 41-7.

Eisenbrand, G., Schmähel, D., Preussmann, R. (1980) Carcinogenicity of N-nitroso-3-hydroxypyrrolidine and doseóresponse study with N-nitrosopiperidine in rats. *IARC. Sci. Publ.* **31** : 6576666.

Fan, A.M. (2011). Nitrate and Nitrite in Drinking Water: A Toxicological Review. **In**: Encyclopedia of Environmental Health, pp. 1376145. Jerome O. Nriagu, Oakland.

Fan, A. M., Willhite, C. C., and Book, S. A. (1987). Evaluation of the nitrate drinking water standard with reference to infant methemoglobinemia and potential reproductive toxicity. *Regul. Toxicol. Pharmacol.* **7** : 135-48.

Food Additives (2008). Health Canada

<http://www.hc-sc.gc.ca/fn-an/securit/addit/index-eng.php>

Accessed on February 2013

Gangolli, S. D., van den Brandt, P. A., Feron, V. J., Janzowsky, C., Koeman, J. H., Speijers, G. J., Spiegelhalder, B., Walker, R., and Wisnok, J.S.(1994). Nitrate, nitrite and *N*-nitroso compounds. *Eur. J. Pharmacol.* **292** : 1-38.

Grohs, B.-M., and Kunz, B.(2000). Use of spice mixtures for the stabilisation of fresh portioned pork. *Food Control* . **11** : 433-436.

Gutierrez, J., Barry-Ryan, C., and Bourke, P.(2009). Antimicrobial activity of plant essential oils using food model media: Efficacy, synergistic potential and interactions with food components. *Food Microbiol.* **26** : 1426150.

Honikel, K.-O. (2008). The use and control of nitrate and nitrite for the processing of meat products. *Meat Sci.* **78** : 68676.

Jayaprakasha, G. K., Selvi, T., and Sakariah, K. K. (2003). Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. *Food Res Int.* **36** : 1176122.

Jensen, F.B. (2003). Nitrite disrupts multiple physiological functions in aquatic animals. *Comp. Biochem. Physiol., Part A: Mol. Integr. Physiol.* **135** : 9624.

Janssen, L. H. J. M., Visser, H., and Roemer, F. G. (1989). Analysis of large scale sulphate, nitrate, chloride and ammonium concentrations in the Netherlands using an aerosol measuring network. *Atmos. Environ.* **23** : 278362796.

Jayatilaka, A., Poole, S. K., Poole, C. F., and Chichila, T. M. (1995). Simultaneous micro steam distillation/solvent extraction for the isolation of semivolatile flavor compounds from cinnamon and their separation by series coupled-column gas chromatography. *Anal Chim Acta.* **302** :1476162.

Kanagal, S., Nagarajan, S., and Lingamallu, J. M. R. (2011). Cumin (*Cuminum cyminum* L.) Seed Volatile Oil: Chemistry and Role in Health and Disease Prevention. In: Nuts and Seeds in Health and Disease Prevention, pp. 4176427. Victor R. Preedy, V.R., Watson, R.R., and Patel, V.B., London, Tucson.

Kapoor, V. K., Chawla, A. S., Manojkumar, and Pradeepkumar (1993). Search for antiinflammatory agents. *India Drugs*. **30** : 481-493.

Kim, I.S., Yang, M-R., Lee, O.-H., and Kang, S.-N. (2011). Antioxidant activities of hot water extracts from various spices. *Int J Mol Sci*. **12**: 4120-4131.

Kurokawa, M., A Kumeda, C., Yamamura, J.-i., Kamiyama, T., and Shiraki, K. (1998). Antipyretic activity of cinnamyl derivatives and related compounds in influenza virus-infected mice Original Research Article. *Eur. J. Pharmacol.* **348** : 45-51.

Lee, E. B., Shin, K. H., Woo, W. S. (1984). Pharmacological Study of Piperine. *Arch. Pharm. Res.* **7** : 127-132.

Lewis, Y. S., Krishnamurthy, N., Nambudiri, E. S., Sankarikutty, B., Shivshankar, and A. S., Mathew, A. G. (1976). The need for growing pepper cultivars to suit pepper products. Proceedings of the International Seminar on Pepper.

Lundberg, J., Gladwin, M. T., Ahluwalia, A., Benjamin, N., Bryan, N. S., Butler, A., Cabrales, P., Fago, A., Feelisch, M., Ford, P. C., Freeman, B. A., Frenneaux, M., Friedman, J., Kelm, M., Kevil, C.G., Kim-Shapiro, D.B., Kozlov, A.V., Lancaster, J.R.J., Lefer, D.J., McColl, K.,

McCurry, K., Patel, R. P., Petersson, J., Rassaf, T., Reutov, V. P., Richter-Addo, G. B., Schechter, A., Shiva, S., Tsuchiya, K., van Faassen, E. E., Webb, A. J., Zuckerbraun, B. S., Zweier, J. L., and Weitzberg, E. (2009). Nitrate and nitrite in biology, nutrition and therapeutics. *Nat. Chem. Biol.* **5** : 865-869.

Matan, N., Rimkeeree, H., Mawson, A. J., Chompreeda, P., Haruthaithanasan, V., and Parker, M. (2006). Antimicrobial activity of cinnamon and clove oils under modified atmosphere conditions. *Int J Food Microbiol.* **107** : 180-185.

Meah, M. N., Harrison, N., and Davies, A. (1994). Nitrate and nitrite in foods and the diet. *Food Addi. Contam.* **11** : 519-532.

Menon, K.V. and Garg, S.R. (2001). Inhibitory effect of clove oil on *Listeria monocytogenes* in meat and cheese. *Food Microbiol.* **18** : 647-650.

Mueller, R. L., Hagel, H. J., Wild, H., Rupp, H., and Domschke, W. (1986). Nitrate and nitrite in normal gastric juice. Precursors of the endogenous N-nitroso compound synthesis. *Oncology*, **43** : 506-53.

Moigradean, D., Lazureanu, A., Poiana, M.-A., Harmanescu, M., Gogoasa, I., and Gergen, I.. (2008). The influence of mineral fertilization about nitrogen content in soil, plant and tomato

fruit. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj Napoca Horticulture*. **65** : 172-177.

O'Leary, M., Rehm, G., and Schmitt, M. (1994). Understanding Nitrogen in Soils. *WW-03770-GO*

Omar, A. A., Artime, E., and Webb, A. J. (2012). A comparison of organic and inorganic nitrates/nitrites. *Nitric Oxide*. **26** : 2296240.

Ouattara, B., Simard, R. E., Holley, R. A., Piette, G. J.-P., and Bégin, A. (1997). Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. *Int. J. Food Microbiol.* **37** : 1556162.

Oussalah, M., Caillet, S., Saucier, L., and Lacroix, M. (2007). Inhibitory effects of selected plant essential oils on four pathogen bacteria growth: *E. coli* O157:H7, *Salmonella typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*. *Food Control*. **18** : 414-420.

Oussalah, M., Caillet, S., Saucier, L., and Lacroix, M. (2007). Inhibitory effects of selected plant essential oils on *Pseudomonas putida* growth, a bacterial spoilage meat. *Meat Sci.* **73** : 236-244.

Pannala, A.S., Mani, A. R., Spencer, J. P. E., Skinner, V., Bruckdorfer, K. R., Moore, K. P., and Rice-Evans, C. A. (2003). The effect of dietary nitrate on salivary, plasma, and urinary nitrate metabolism in humans. *Free Radic. Biol. Med.* **34** : 576-584.

Pearson, A. M., and Gillett, T. A. (1996). Processed Meats. Chapman and Hall, New York, NY

Pegg, R.B., and Shahidi, F. (2000). Nitrite Curing of Meat. The N-Nitrosamine Problem and Nitrite alternatives. Food and Nutrition Press, Inc., Trumbull, CT.

Perumalla, A. V. S., and Hettiarachchy, N. S.(2011). Green tea and grape seed extracts Potential applications in food safety and quality. *Food Res. Int.* **44** : 827-839.

Petersen, A., and Stoltze, S. (1999). Nitrate and nitrite in vegetables on the Danish market: Content and intake. *Food Addi. Contam.* **16** : 291-299.

Pokorny, L., and Maturana, I., Bortle, W. H. (2006). Sodium nitrate and nitrite. **In:** Kirk-Othmer Encyclopedia of Chemical Technology, John Wiley and Sons, 5th Ed, New York, [online version].

Prabhakaran Nair, K.P. (2004). Advances in Agronomy, The Agronomy and Economy of Black Pepper (*Piper nigrum* L.) ô The ôKing of Spicesö. **In** : Agronomy and Economy of Black Pepper and Cardamom, pp 2716389. Prabhakaran Nair, K.P., New Delhi.

Pradsad, M. M., Seenayya, G. Effect of spices on the growth of red halophilic cocci isolated from salt cured fish and solar salt. *Food Res. Int.* **33** : 7936798.

Prospero, M., and Savoie, D. L. (1989). Nitrate in the Atmospheric Boundary Layer of the Tropical South Pacific: Implications Regarding Sources and Transport. *J. Atmos. Chem.* **8** : 391-415.

Sallam, K. I., Ishioroshi, M., and Samejima, K. (2004). Antioxidant and Antimicrobial Effects of Garlic in Chicken Sausage. *Lebenson Wiss Technol.* **37** : 849-855.

Omar, S. A., Arttime, E., and Webb, A. J. (2012). A comparison of organic and inorganic nitrates/nitrites Original Research Article. *Nitric Oxide.* **26** : 229-240.

Rosengarten, F. Jr. (1973). The Book of Spices. Pyramid Books, New York.

Shaikh, J., Bhosale, R., and Singhal, R. (2006). Microencapsulation of black pepper oleoresin. *Food Chem.* **94** : 1056110.

Sebranek, J. G. (2009). Basic curing ingredients. **In** : Ingredients in Meat Products, pp. 1-24.

Tarte, R. Eds. Springer Science+Business Media LLC, New York.

Sebranek, J. G., and Bacus, J. N. (2007). Cured meat products without direct addition of nitrate or nitrite: What are the issues? *Meat Sci.* **77** :1366147.

Shaath, N. A., and Azzo, N. R. (1993). Essential oil of Egypt. **In** : Food flavor ingredients and composition, pp. 5916603. Charalambous, G., Eds., Elsevier Sci. Pub, Amsterdam.

Shirin, A. P. R., and Prakash, J. (2010). Chemical composition and antioxidant properties of ginger root (*Zingiber officinale*). *J. Med. Plants Res.* **4** : 2674-2679.

Sofos, J.N., et al. (1979). Botulism control by nitrite and sorbate in cured meats. *J. Food Prot.* **42** : 739-770.

Stoilova, I., Krastanov, A., Stoyanova, A., Denev, P., and Gargova, S. (2007). Antioxidant activity of a ginger extract (*Zingiber officinale*). *Food Chem.* **102** : 7646770.

Tabak, M., Armon, R., and Neeman, I. (1999). Cinnamon extractsø inhibitory effect on *Helicobacter pylori* Original Research Article. *J. Ethnopharmacol.* **67** : 269-277.

Tajkarimi, M.M., Ibrahim, S. A., and Cliver, D. O. (2010). Antimicrobial herb and spice compounds in food. *Food Control.* **21** : 119961218.

Toghyani, M., Gheisari, A., Ghalamkari, G., and Eghbalsaied, S. (2011). Evaluation of cinnamon and garlic as antibiotic growth promoter substitutions on performance, immune responses, serum biochemical and haematological parameters in broiler chicks. *Livest Sci.* **138** : 167-173.

Thomson, B. (2004). Nitrites and nitrates dietary exposure and risk assessment. Christchurch Science Centre Christchurch, NZ

Townsend, W. E., and Olson, D. G. (1987). Cured meats and cured meat products processing. In *The Science of Meat and Meat By Products*, pp. 1936216, 4316456. Price, J. F., and Schweigert, B. S., Eds., Food and Nutrition Press Inc., Westport.

Tu, X., Xiao, B., Xiong, J., and Chen, X.. (2010). A simple miniaturised photometrical method for rapid determination of nitrate and nitrite in freshwater. *Talanta.* **82** : 9766983.

U.S. Department of Agriculture. (1995). Processing inspectors' calculation shandbook. Available at: <http://www.fsis.usda.gov/OPPDE/rdad/FSISDirectives/7620-3.pdf>. Accessed December 8, 2009.

U.S EPA (2006). Toxicity and Exposure Assessment for Children's Health.
http://www.epa.gov/teach/chem_summ/Nitrates_summary.pdf .

Accessed on February, 2013

Vasavada, M. N., and Cornforth, D. P. (2005). Evaluation of milk mineral antioxidant activity in meat balls and nitrite-cured sausage. *J. Food Sci.* **70** : 2506253.

Viani, F., Siegrist, H. H., Pignatelli, B., Cederberg, C., Idström, J. P., Verdu, E. F., Fried, M., Blum, A. L., and Armstrong, D. (2000). The effect of intra-gastric acidity and flora on the concentration of Nnitroso compounds in the stomach. *Eur. J. Gastroenterol. Hepatol.* **12** : 165-73.

World Health Organization (2004). Rolling Revision of the WHO Guidelines for Drinking-Water Quality. Nitrates and nitrites in drinking-water.

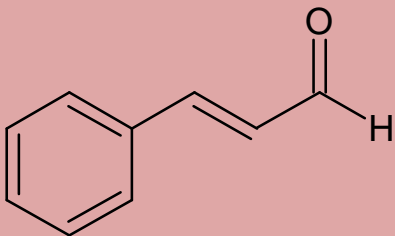
Yadav, A. S., and Singh, R. P.(2004). Natural preservatives in poultry meat. *Arch. Microbiol.* **181** : 861.

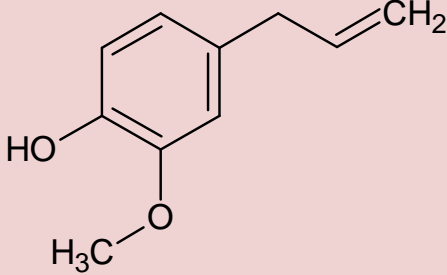
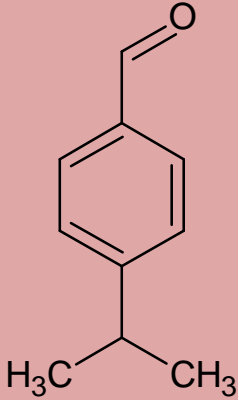
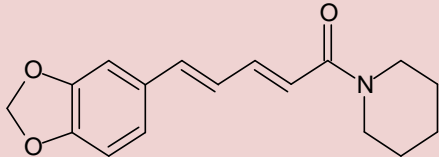
Yocom, J. E. (1982). Indoor/outdoor air quality relationships: a critical review. *JAPCA J Air Waste Ma.* **32** : 5006606.

Zaika, L. L., Kissinger, J. C. (1978). Effect of major spices in Lebanon bologna on production by starter culture organisms. *J. Food. Prot.* **41** : 429-431.

Zheng, W., and Wang, S. Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *J Agric Food Chem.* **49** : 5165-5170.

Table 1: Summary of the properties of spices

	Major constituent	properties	References
Cinnamon <i>(Cinnamomum zeylanicum)</i>	Cinnamaldehyde 	antimicrobial, antifungal anti-inflammatory, anti-allergenic, anti-ulcerogenic, anti-pyretic, anaesthetic	(Delespaul et al., 2000), (Chang et al., 2001), (Kim et al., 2004), (Kurokawa et al., 1998), (Qin et al., 2009), (Lin et al., 2003), (Okawa et al., 2001), (Toda, 2003), (Inouye et al., 2001), (Smith-Palmer et al., 1998)
	4-allyl-2-methoxyphenol	anti-inflammatory	(Tajkarimi,

<p>Cloves <i>(Eugenia Caryophyllus)</i></p>	<p>(eugenol)</p> 	<p>analgesic effect, antiplatelet, antiviral, flavoring properties bacterial inhibition antimicrobial activity</p>	<p>2010) (Menon and Garg, 2001)</p>
<p>Cumin <i>(Cuminum cyminum L.)</i></p>	<p>Cumin aldehyde</p> 	<p>anti-bacterial antioxidant, hypoglycemic, hypolipidemic activities anti-tumor anti-diabetic diuretic anti-bacterial</p>	<p>(Sahana, 2011) (El-Daly, 1998) (Al-Hader et al., 1993), (Zaoui et al., 2000) (Kamali et al., 1998)</p>
<p>Black pepper <i>(Pepper Nigrum)</i></p>	<p>Piperine</p> 	<p>analgesic antipyretic antioxidant antimicrobial</p>	<p>(Kapoor et al., 1993), (Lee et al., 1984)</p>

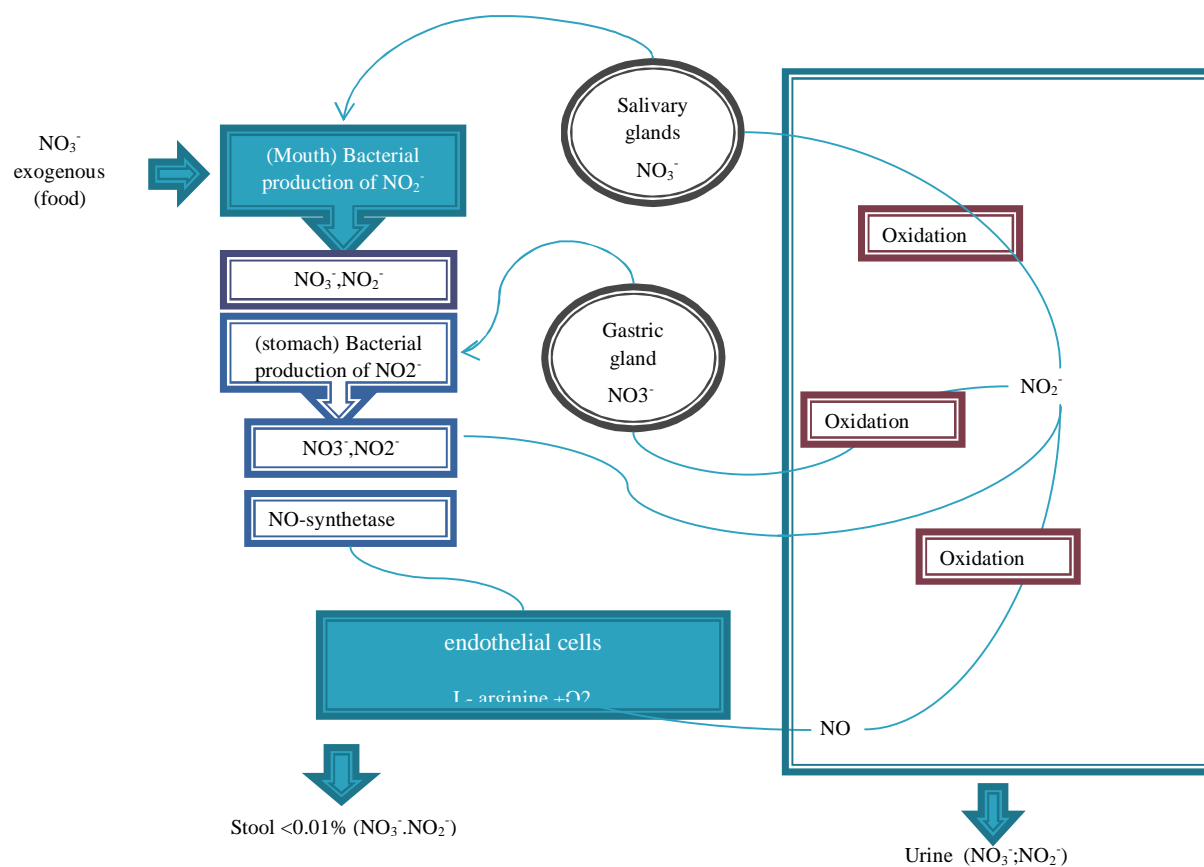


Figure 1: Endogenous biosynthesis of nitrate

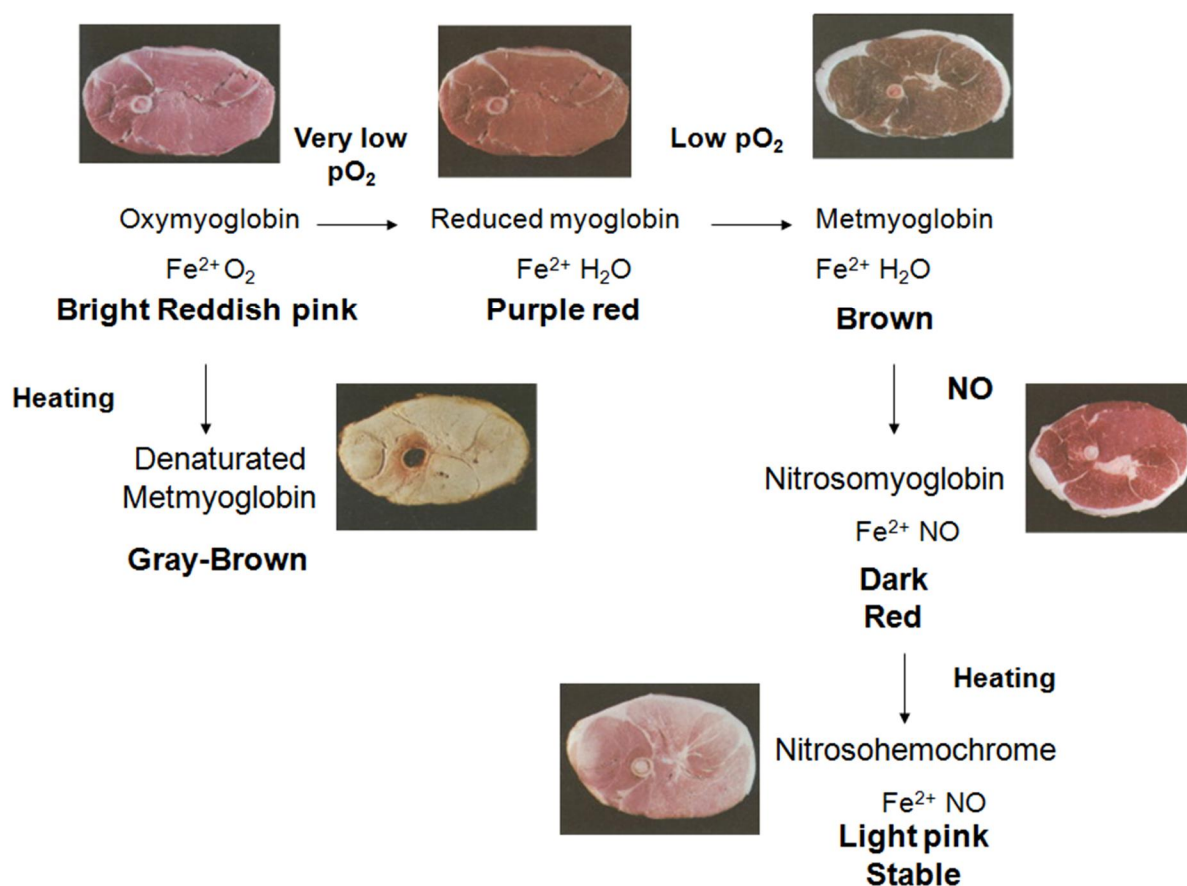


Figure 2: Transformation of myoglobin in meat

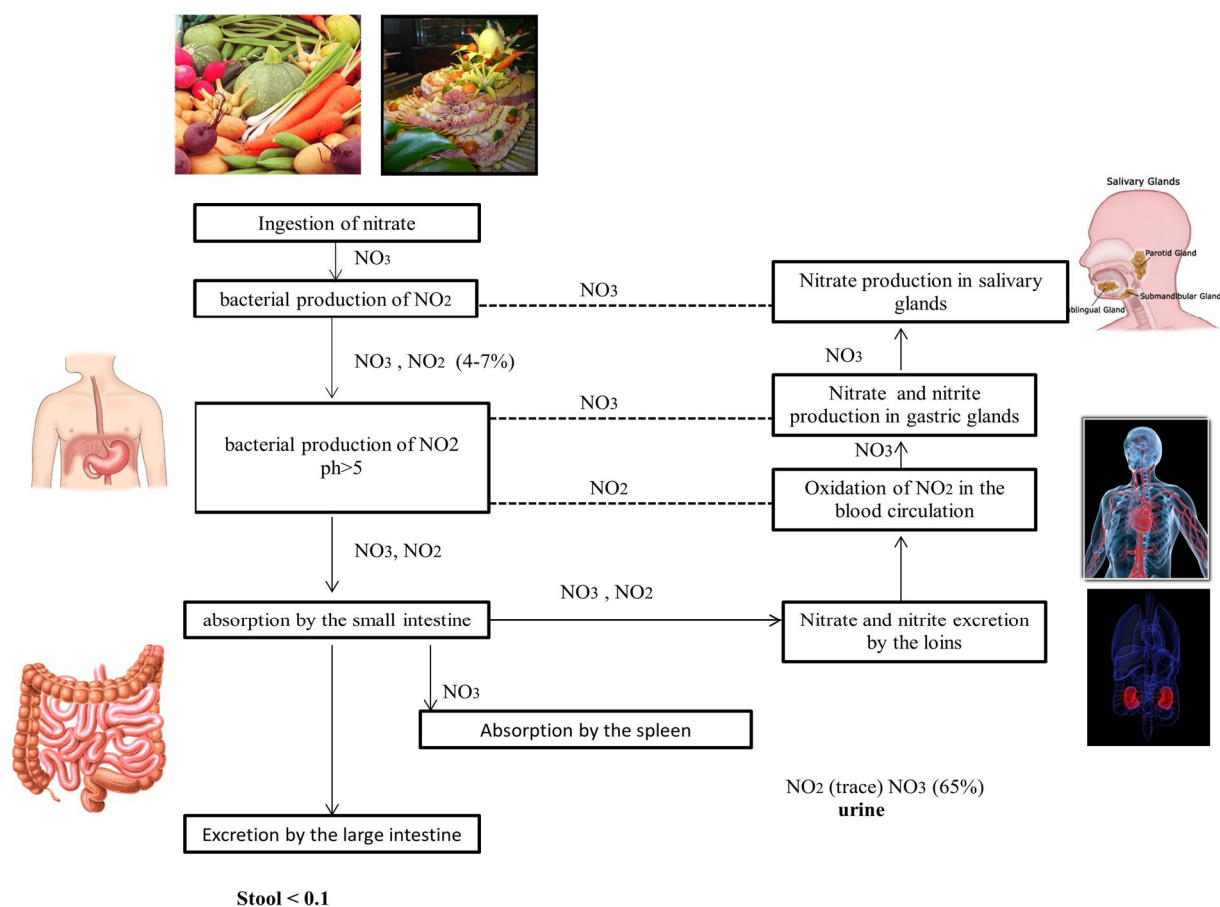


Figure 3: Toxicokinetics of nitrates and nitrites in human body

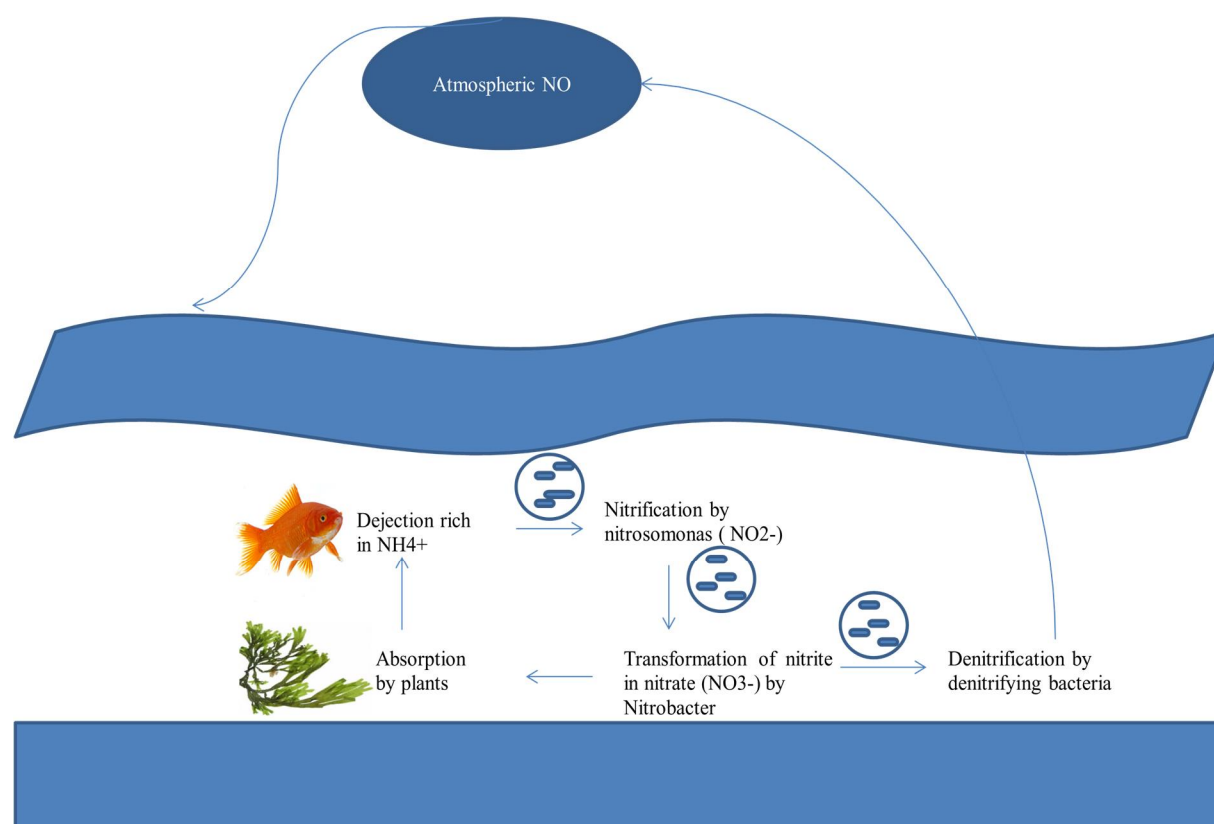


Figure 4 : Nitrate cycle in water

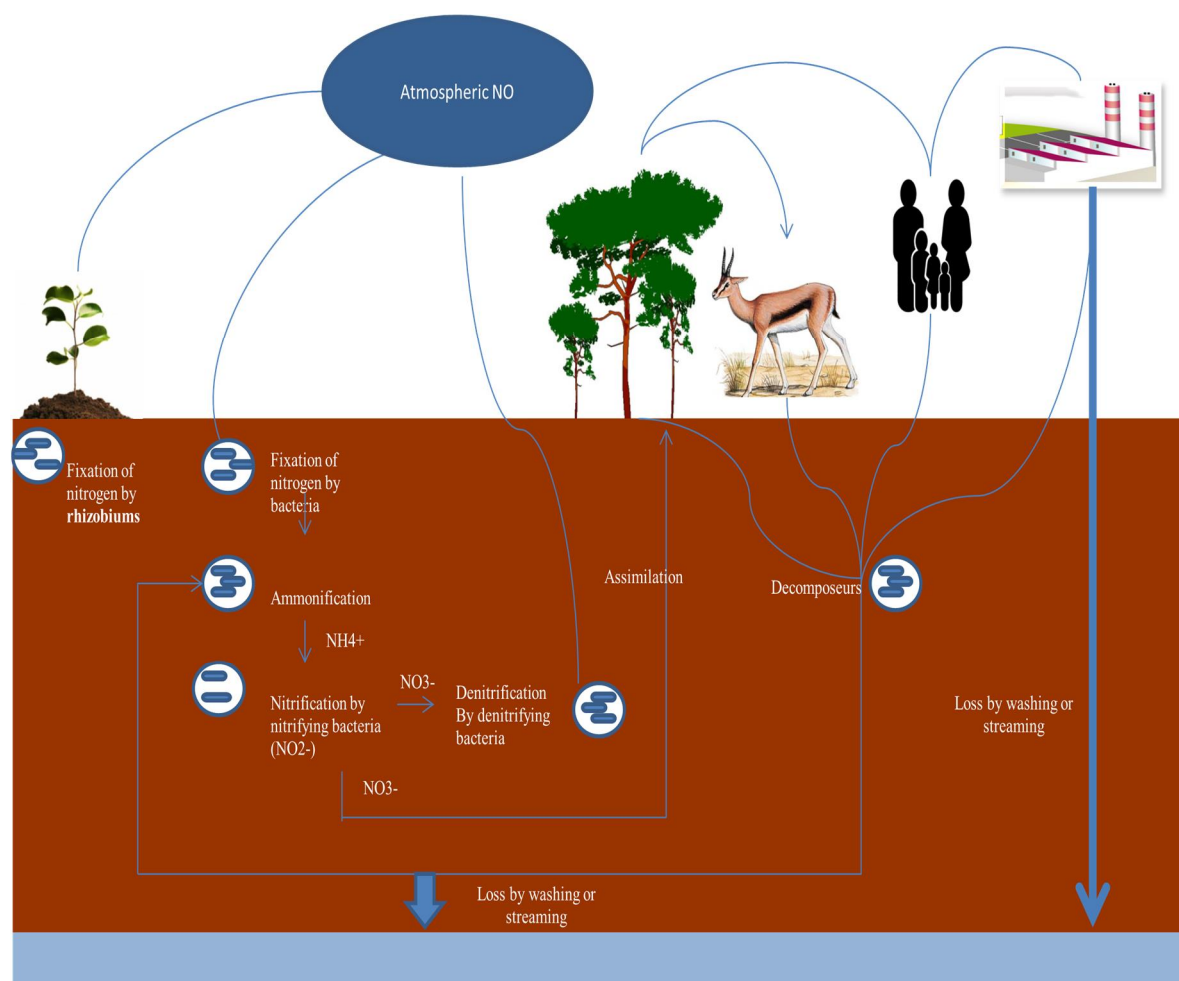


Figure 5: Nitrogen cycle