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## ■ Nitrates and Nitrites in meat products

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## ■ Νιτρικά και νιτρώδη άλατα στα προϊόντα του κρέατος

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**ABSTRACT.** Nitrates and nitrites have been traditionally used as curing agents in the production of cured meat products. Beneficial effects of the addition of nitrates and nitrites to meat products are the improvement of quality characteristics as well as the microbiological safety. The nitrates and nitrites are mainly responsible for the development of the distinct flavor, the stability of the red color, as well as the protection against lipid oxidation in cured meat products. The nitrites show important bacteriostatic and bacteriocidal activity against several spoilage bacteria as well as foodborne pathogens found in meat products. The nitrites prevent the growth and toxin production by *Clostridium botulinum*.

According to Commission Regulation (EU) No. 1129/2011, nitrates (sodium nitrate, E251; potassium nitrate, E252) and nitrites (potassium nitrite, E249; sodium nitrite, E250) are listed as permitted food additives. Nitrates are relatively non-toxic, but nitrites, and nitrites metabolic compounds such as nitric oxide and N-nitroso compounds, have raised concern over potential adverse health effects. Recently, the International Agency for Research on Cancer (IARC) concluded that ingested nitrates or nitrites are probable carcinogen to humans under conditions favoring the endogenous nitrosation. Legal limits for the addition of nitrates and nitrites have been set by several countries and EU [Commission Regulation (EU) No. 601/2014]. Several data from recent reviews conducted in several countries on the levels of nitrates and nitrites in cured meat products were summarized. In recent reviews, the residual levels of nitrites in cured meat samples have been constantly reduced and are in accordance with the legal limits set by most countries.

**Keywords:** nitrates, nitrites, meat products, *Clostridium botulinum*.

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**ΠΕΡΙΛΗΨΗ** Τα νιτρικά και νιτρώδη άλατα χρησιμοποιούνται εκτεταμένα στην βιομηχανία του κρέατος καθώς συμβάλλουν σημαντικά τόσο στην βελτίωση των ποιοτικών χαρακτηριστικών, όσο και στην ασφάλεια των προϊόντων του κρέατος. Στα προϊόντα αυτά τα νιτρικά και νιτρώδη άλατα συμβάλλουν στην σταθερότητα του ερυθρού χρώματος, στην ανάπτυξη και διατήρηση του ιδιαίτερου αρώματος, αλλά και στον έλεγχο της οξειδωσης των λιπών. Τα άλατα αυτά εμφανίζουν επίσης σημαντική βακτηριοστατική και βακτηριοκτόνο δράση έναντι αλλοιογόνων αλλά και σημαντικών παθογόνων μικροοργανισμών στα προϊόντα του κρέατος. Από τις πιο σημαντικές δράσεις τους είναι η αναστολή της ανάπτυξης και της παραγωγής τοξίνης από το *Clostridium botulinum*.

Σύμφωνα με το Κανονισμό (ΕΕ) αριθ. 1129/2001 τα νιτρικά [νιτρικό νάτριο (E251), νιτρικό κάλιο (E252)] και νιτρώδη άλατα [νιτρώδες κάλιο (249), νιτρώδες νάτριο (E250)] περιλαμβάνονται στα εγκεκριμένα πρόσθετα τροφίμων. Τα νιτρικά θεωρούνται σχετικά μη τοξικές ενώσεις αλλά τα νιτρώδη και τα μεταβολικά παράγωγά τους, όπως το μονοξειδίο του αζώτου και διάφορες N-νιτροζο-ενώσεις, έχουν συνδεθεί με δυσμενείς επιδράσεις στην υγεία του ανθρώπου. Ο Διεθνής Οργανισμός Έρευνας για τον Καρκίνο (International Agency for Research on Cancer, IARC, 2010) κατέληξε στο συμπέρασμα ότι τα νιτρικά και νιτρώδη είναι πιθανά καρκινογόνες ουσίες για τον άνθρωπο υπό συνθήκες που ευνοούν τον ενδογενή σχηματισμό N-νιτροδο ενώσεων. Τα όρια για τα νιτρικά και νιτρώδη άλατα στα προϊόντα του κρέατος έχουν θεσμοθετηθεί από την Ευρωπαϊκή νομοθεσία [Κανονισμός (ΕΕ) αριθ. 601/2014] για τα τρόφιμα, αλλά και σε άλλες χώρες παγκοσμίως. Από τα αποτελέσματα ερευνών που έγιναν σε διάφορες χώρες διαπιστώνεται ότι τα επίπεδα των νιτρικών και νιτρωδών αλάτων στα προϊόντα κρέατος μειώνονται συνεχώς και στην πλειονότητα τους είναι σύμφωνα με τα όρια που έχουν οριστεί στις αντίστοιχες χώρες.

**Λέξεις ευρετηρίασης:** νιτρικά, νιτρώδη, προϊόντα κρέατος, *Clostridium botulinum*.

## INTRODUCTION

Nitrates (potassium nitrate,  $\text{KNO}_3$ ; sodium nitrate,  $\text{NaNO}_3$ ) and nitrites (potassium nitrite,  $\text{KNO}_2$ ; sodium nitrite,  $\text{NaNO}_2$ ) are well known as curing agents in cured meat production. Nitrates and nitrites along with sodium chloride are commonly added to meat during curing meat process. Nitrates and nitrites, as additives, contribute to the cured meat flavor, inhibit the growth of microorganisms, particularly *Clostridium botulinum*, and effectively control rancidity by inhibiting lipid oxidation (Pearson and Gillett, 1996). The beneficial effects of nitrates and nitrites were early recognized, since they are usually found in the salt, used for meat curing.

Nitrates and nitrites are present in several food products, as naturally occurring compounds and thus nitrates and nitrites form part of the human diet. They are also well-known food additives listed as official preservatives according to Commission regulation (EC) No. 1129/2011.

The potential dangerous effect on the health of nitrates and nitrites was discovered in the 1960s and a debate on safety of cured meat products was initiated, since nitrates and nitrites are the common

additives used in cured meat products. This debate was largely focused on cured meat products, although the potable water and vegetables are also considered as substantial sources of nitrates intake. The major concern of nitrates/nitrites in food is related to the potential ability of nitrites to form carcinogenic N-nitroso compounds.

The first half of the 20th century brought a gradual shift from nitrates to nitrites addition in cured meat products, due to a faster curing time, an increased production capacity and the better knowledge of nitrite chemistry. Nowadays, nitrates are rarely used, except a few special products such as dry cured hams and dry sausage because of their slow curing process (Sebranek and Bacus 2007).

The present work is aimed to review the nitrates and nitrites use in cured meat products, the adverse health effect of nitrates and nitrites consumption, as well as recent published data on surveys conducted on the nitrates/nitrites content of meat products.

## FOOD SOURCES OF NITRATES AND NITRITES

The total nitrates intake in humans mainly derives

from food (80%) and water (14%) (Archer, 2002; WHO, 2007).

Although the different lifestyles and dietary habits in various countries can lead to wide variations in the amount of consumed nitrates, the World Health Organization estimates that the mean daily dietary intake of nitrates is usually ranged from 43 to 141 mg (WHO, 2007).

Several works indicate that the majority of the nitrates intake is attributed to plant foods. Approximately 98% of the nitrates intake from food is attributed to fruits and vegetables, while only the remaining 2% to cured meat products (Larsson et al., 2011). The National Academy of Sciences (NAS, 1981) reported that 87% of the dietary nitrates intake is associated with the consumption of vegetables. Several factors (e.g. nitrates uptake, nitrates reductase activity, growth rate and growth conditions, processing methods such as heat treatments and storage conditions) affect the ultimate nitrates content of vegetables. A factor of great importance is the application of fertilizers generally resulting in a greater uptake of nitrogen in vegetables and eventually in higher nitrates content. Leafy vegetables such as lettuce and spinach tend to have higher levels of nitrates than seeds or tubers (EFSA 2008).

In contrast to nitrates, nitrites are mainly formed from the endogenous nitrates conversion. Dietary nitrites represent less than 20 % of the total daily nitrites exposure, the remaining 80 % resulting from the endogenous bioconversion of dietary nitrates to nitrites in saliva. Humans generally consume 1.2–3.0 mg of nitrites per day (WHO, 2007). Among consumed food, the total daily nitrites intake may be as high as 4% and 19% for fresh meat and cured meat, respectively (MAFF, 1998). Vegetables represent only 19% of the total daily nitrites intake (WHO, 2007). According to Archer (2002) cured meat products comprise 4.8% of daily nitrites intake and vegetables account for just 2.2%.

## **THE NITRATES AND NITRITES CHANGES IN CURED MEAT PRODUCTS**

The nitrates are inert compounds and should be

reduced to nitrites, which are the highly active curing compounds. The reduction of nitrates to nitrites is mainly accomplished by either naturally present bacteria in the meat or by the addition of bacteria possessing a nitrates reductase activity (Sebranek and Bacus, 2007; Hammes 2012). The most efficient nitrates reducing organisms are staphylococci and micrococci (Gøtterup et al., 2008), which also affect the fermentation process in meat products. Lactic acid bacteria can also reduce nitrates to nitrites but their potential has not been used in commercial starter cultures. Although it is generally accepted that the nitrates reduction is exclusively performed by microorganisms, nitrates reductase activity is normally present in animal physiology (Hammes, 2012). Furthermore, as reported by Honikel (2008), nitrates reduction has been also observed in heated cured meat products, which may be due to chemical reactions.

The rate of nitrates reduction varies in different meat products. In certain traditional products, nitrates act as a reservoir of nitrites, the rate of reduction being dependent on the nature of the natural microbial flora. Occasionally nitrates can be also detected in meat products processed with nitrites additives, as the result of microbial activity (EFSA, 2003).

In meat, nitrites are usually decreased rapidly and converted to a variety of compounds such as nitrous acid, nitric oxide (NO) and nitrates (Honikel, 2004). At a pH of the meat, generally 5.6 to 5.8, nitrites are mostly present in the dissociated form, and are converted to several intermediate compounds, which are unstable and therefore difficult to be determined. The compounds derived from nitrites can act as oxidizing, reducing or nitrosylating agents (Honikel, 2004).

The decrease of nitrites in meat products may be due to the action of meat endogenous reducing substances, e.g. sulphurcontaining amino acids (cysteine), or to additives such as ascorbate. In the last decades, ascorbic acid or ascorbate, isoascorbate (erythorbate) have been used as additives in cured meat batters. The ascorbate reacts with oxygen, by forming dehydroascorbate, and reduces the amount of nitrites which could be oxidized to nitrates (Honikel, 2008).

Ascorbate seems also to react with resulting NO; the so formed new NO compounds can further react with other meat ingredients (Izumi et al., 1989).

It is generally accepted that the formation of NO from nitrites is a prerequisite step for most meat curing reactions (Møller and Skibsted, 2002). NO reacts in various ways, concurrent with one another. The decrease in the NO amount is due to NO reactions with myoglobin and other substrates present in meat, eg amino acids such as cysteine or glutathione (Reddy et al., 1983; Honikel, 2008).

Consequently many of the nitrites added in the meat are decreased through a series of NO reactions during manufacture and storage of cured meat products. The rate of nitrites loss depends on many factors such as the temperature of the heat process, the pH of the product, the storage temperature and the addition of ascorbic acid or other reducing agents (EFSA, 2003). An amount of 10% to 20% of the originally added nitrites is typically present after the manufacturing process (Perez-Rodriguez et al., 1996; Cassens, 1997). This amount of nitrites, referred to as residual nitrites, slowly decline over the storage life of the cured meat products, and sometimes reach non-detectable levels (Sindelar and Milkowski, 2012). In most meat products, there is no simple and direct relationship between the initial amount and the residual nitrites amount (EFSA, 2003).

## **BENEFICIAL EFFECTS OF NITRATES OR NITRITES ADDITION IN MEAT PRODUCTS**

The use of nitrates and nitrites as curing agents has beneficial effects on the quality factors as well as on the microbiological.

### **Quality effect**

The addition of nitrates and nitrites in cured meat shows a positive effect on quality meat characteristics such as color, flavor or stability against oxidation.

The color of fresh meat is red to shaded pink, which is an extremely important attribute for consumer acceptance (Cornforth and Jayasingh, 2004). The basic color component of meat is myoglobin, which

is a globular protein consisting of an heme group with a centrally located atom of iron (Fe) surrounded by a globin moiety. According to the iron oxidation state ( $Fe^{2+}$  or  $Fe^{3+}$ ) the myoglobin may be found in muscles in three states (myoglobin, oxymyoglobin and metmyoglobin). Originally, myoglobin ( $Fe^{2+}$ ) does not bind any ligand. After slaughtering of the animal, the oxygen in direct contact with the muscles can bind  $Fe^{2+}$  of myoglobin and form oxymyoglobin, which is red in color but not very stable. However,  $Fe^{2+}$  may further oxidized to  $Fe^{3+}$  by oxidizing compounds such as oxygen or nitrites. Thus, this oxidation in the presence of oxygen leads to formation of metmyoglobin ( $Fe^{3+}$ ), which is brown in color, and it is also a less desirable attribute to consumers.

In cured meat, the red color is developing in a number of complicated reaction steps until the characteristic curing red pigment, is formed. NO deriving from nitrites, is bound to the iron ion ( $Fe^{+2}$ ) located in the centre of the porphyrin ring system of myoglobin and forms NO-myoglobin (nitrosomyoglobin), which is colored but not stable. Nitrosomyoglobin can be transformed to the characteristic stable red pigment of cured meat products (nitroso-myochromogen), either by the application of heat in the heat processed meat products or in the low acid environment of fermented meat products. In heat processed meat products, the protein moiety of the nitrosomyoglobin is denatured, but the red NO-porphyrin ring system (nitroso-myochromogen) which is stable, still exists and is found in meat products heated up to 120°C (Honikel, 2008). In low acid fermented meat products, the protein moiety of nitrosomyoglobin is also denatured but the nitroso-myochromogen is not affected and improves the color stability. It is generally accepted that a small amount (10-15 ppm) of residual nitrites is required for the color stability in cured meat products (Houser et al., 2005). The role of nitrites in the cured meat flavor is a quite complex procedure and not fully understood. Meat curing practices not only involve sodium nitrite and salt, but also sugar, reducing agents, phosphates and spices, which also contribute in the flavor. However, they are nitrites that are mainly responsible for the development of the distinct flavor

of cured meat products. Since nitrites are bound with proteins and lipids, several volatile and not volatile compounds are formed and contribute to the flavor of the cured meat (Jira, 2004; Sebranek and Bacus, 2007). Binding of nitrites to sulfur containing amino acids of meat proteins results in the production of SH-residues with specific aroma and flavor (Hammes, 2012). Many studies indicated the difficulties of isolating the flavor compounds of cured meat products (Olesen et al., 2004; Sindelar and Milkowski, 2012). Several flavor compounds have been isolated in cured meat products, such as hydrocarbons, alcohols, ketones, furans, pyrazines, and sulfur- and nitrogen- containing heterocyclic compounds. Pegg and Shahidi, (2000) identified an astonishing number of 135 volatile compounds in nitrites-cured ham.

Nitrites exhibit antioxidant activity, and slow the breakdown of unsaturated fatty acids, as well as the development of secondary oxidation flavor compounds (Shahidi, 1998). The antioxidant effect of nitrites is primarily due to oxygen deletion. The NO molecule itself can easily be oxidized to NO<sub>2</sub> in the presence of oxygen resulting to an oxygen sequestering (Honikel, 2008). Due to lack of oxygen, the development of rancidity or a warmed off flavor are retarded in cured meat products.

Moreover, a stable complex between heme-bound iron and nitrites is formed, which inhibits a release of iron ions. Consequently, free iron ions (Fe<sup>2+</sup>) are not available for the initiation of lipid peroxidation (Andrée et al., 2010). Furthermore, nitrites are also able to react with free iron ions and form a complex. It is also assumed that nitrites are able to stabilize polyunsaturated fatty acids forming nitro-nitroso derivatives (Freybler et al., 1993). As a consequence, cell membranes are protected against lipid peroxidation and the stability of cured meat product during storage is enhanced. The addition of nitrites to meat products also leads to lower contents of harmful cholesterol oxides (Arneth and Münch, 2002). In addition, nitrites can form various nitroso- and nitrosyl compounds, like S-nitrosocysteine, which exhibit antioxidant properties (Shahidi, 1992).

Nitrites at relatively low levels (40 ppm) proved

to inhibit warmed off flavor development (Al-Shuibi and Al-Abdullah 2002). Because nitrites are highly effective as antioxidants, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are not usually used in the majority of the cured meat products (Sindelar and Milkowski, 2012).

### **Microbiological safety**

Nitrites exhibit important bacteriostatic and bacteriocidal activity against several spoilage bacteria as well as foodborne pathogens found in meat products (Sindelar and Milkowski, 2012). However, the majority of studies concerning the antimicrobial properties of nitrites have been focused on *C. botulinum* in several meat products produced in various countries (EFSA, 2003).

Nitrites show antibotulinal activity against both vegetative cells as well as spores of the pathogen in thermally processed meat products (Pierson and Smooth 1982). Despite the vast amount of research, the precise nitrites action against *C. botulinum* or other bacteria has not absolutely clarified in meat products.

The antimicrobial action of nitrites is likely attributed to reactions associated with the generation of NO or nitrous acid (Møller and Skibsted, 2002). Reddy et al. (1983) suggested that the inactivation of iron-sulphur proteins of *C. botulinum* vegetative cells by NO, is the probable mechanism of inhibition of the pathogen in nitrites-cured meat products. The antimicrobial activity of nitrites in cured meat products is also enhanced from other antimicrobial hurdles such as heat treatment, pH, salt, water activity (a<sub>w</sub>), redox potential and other curing ingredients (Tompkin, 2005). Several studies indicated that nitrites inhibit bacteria more effectively at low pH (Roberts, 2005; Allaker et al., 2001). Other components, such as ascorbate or isoascorbate / erythorbate and also cysteine, may enhance the antibotulinum efficacy of nitrites in cured meat by sequestering metal ions (Tompkin et al., 1978). Robinson et al. (1982) has shown that ascorbate in meat products reduces the toxin production by proteolytic *C. botulinum* types A and B together with nitrites and NaCl.

Although in most cured meat products, the nitrites are added to prevent the growth and toxin production by *C. botulinum*, the required amount for this antimicrobial efficacy varies from product to product. According to the opinion of the Scientific Committee on Food of the European Food Safety Authority (EFSA), 50-100 mg nitrites per kg meat may be sufficient for the majority of cured meat products; however, for other meat products, especially those with a low NaCl content and a prolonged shelf life, the presence of a higher content, as high as 150 mg kg<sup>-1</sup> of nitrites, is required for efficacy against *C. botulinum* (EFSA 2010). As nitrites levels increase, inhibition of *C. botulinum* growth and toxin production also increases (Sofos et al., 1979b). The nitrites level required for color development (roughly 25 ppm or less) is quite small as compared to this required for *C. botulinum* control in cured meat (Sofos et al., 1979a).

Antibacterial compounds are also formed as a result of nitrites-related reactions during storage (Hustad et al., 1973). The contribution of the residual amount of nitrites in the microbiological safety of meat products has been also disputed. For example, in meat products containing ascorbate (or isoascorbate / erythorbate) resulting in more rapid loss of nitrites, the residual nitrites content is very low, and sometimes below the level of detection, although the growth of *C. botulinum* is still prevented (EFSA, 2003).

Nitrates have no direct activity against *C. botulinum*. The addition of nitrates is usually required in particular traditional dry meat products, typical of the Mediterranean countries, like long-ripened dry-fermented sausages of high pH and dry-cured ham (EFSA, 2003). In these products nitrates act as reservoirs of nitrites, progressively reduced to nitrites, which contribute not only to sensory properties but also to safety due to their antimicrobial action.

Control measures such as good manufacturing practice (GMP) and hazard analysis critical control point (HACCP) during production of certain meat products could result in reduction of the required levels of nitrates-nitrites, although these products may be not strictly classified as “cured meat products” (EFSA, 2003).

The antimicrobial activity of nitrites against food-borne pathogens in cured meat products has been also studied in recent years (Milkowski et al., 2010). Nitrites have been proved to have a stronger antimicrobial activity against Gram positive than Gram negative bacteria. However, the antimicrobial efficacy of nitrites against Gram negative bacteria is also significant (Pichner et al., 2006).

In an early study, Bayne and Michener (1975) reported no nitrites efficacy against *Staphylococcus* in frankfurters. Contrary, in a later study, Buchanan and Solberg (1972) found a bacteriostatic action on *Staphylococcus aureus* and they suggested that their results provided evidence that the pathogen may be effectively controlled with 200 ppm of nitrites. The antimicrobial action of nitrites against *S. aureus* was also reported by Mahindru (2008).

Tompkin (2005) found that nitrites were effective for controlling Gram-negative enteric pathogens such as *Salmonella* and *Escherichia coli*. Pichner et al. (2006) showed the inhibitory activity of nitrites against enterobacteria and Shigatoxin producing *Escherichia coli* (STEC) in fermented sausages.

Nitrites with sodium lactate and sodium diacetate have been found to act synergistically against *Listeria monocytogenes* in cured meat products (Seman et al., 2002; Gill and Holley, 2003; Legan et al., 2004; USDA, 2006). Milkowski et al. (2010) reported inhibitory activity of nitrites against *L. monocytogenes*, *E. coli* O157:H7, *S. aureus*, and *Bacillus cereus* in cured meat products.

## METABOLISM OF NITRATES AND NITRITES

The metabolism of nitrates in the human body has been extensively studied. Nitrates undergo a number of metabolic conversions, and are recycled between the saliva and the gut and the bile and the gut (EFSA, 2008). Briefly, once nitrates are ingested, they are quickly absorbed in the upper part of the small intestine in humans (Bartholomew and Hill, 1984). Absorbed nitrates are rapidly transported by the blood and selectively secreted by the salivary glands, and probably other exocrine glands. Lundberg et al. (1994) reported that approximately 25% of ingested

nitrates are secreted in saliva. In the oral cavity, the bacteria reduce approximately 20% of the secreted nitrates into nitrites (Lundberg et al., 2004 and 2008), and formed nitrites are then swallowed along with the unconverted nitrates.

Healthy adults have a salivary conversion of nitrates to nitrites of normally 5-7% of the total nitrates intake (Lundberg et al. 1994). In the stomach, nitrites are rapidly transformed to nitrous acid, which in turn is decomposed to nitrogen oxides including NO (McKnight et al., 1997). Most of the absorbed nitrates in the digestive system are ultimately excreted in the urine, but considerable salvage takes place in advance through selective reabsorption from the kidney and the salivary recirculation (Bartholomew and Hill, 1984). In addition to the dietary intake, nitrates are formed endogenously, with NO as the precursor. The main source of endogenous nitrates in mammals is the L-arginine-NO synthase pathway, which is constitutively active in numerous cell types throughout the body. NO is produced from the amino acid L-arginine and molecular oxygen by nitric oxide synthetase (NOS) (Lundberg et al. 2009).

Nitrites can be reduced and form NO in human tissues via several pathways by involving haemoglobin, myoglobin, or xanthine oxidoreductase (Lundberg et al., 2008, 2009). The generation of NO by these pathways is mainly enhanced during hypoxia and acidosis. The proteins modified by NO contribute to physiological hypoxic signaling, vasodilation, cellular respiration and cellular response to ischaemic stress (Lundberg et al. 2009).

### **CONCERNS ASSOCIATED WITH NITRATES AND NITRITES IN MEAT PRODUCTS**

The first international evaluation of the risks associated with the ingestion of nitrates and nitrites was conducted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1961 (FAO/WHO, 1962). Since then, research works have clearly shown that nitrates and nitrites have both acute and long-term effects on human health.

Nitrates per se are relatively non-toxic, but nitrates metabolic products such as nitrites, NO and N-nitroso

compounds (NOCs), have raised concern over potential adverse health effects. The acute toxicity of nitrites is approximately 10-fold higher than that of nitrates. The lethal oral doses for human beings are established in 80–800 mg nitrates kg<sup>-1</sup> bw and 33–250 mg nitrites kg<sup>-1</sup> bw (Schuddeboom, 1993). The high amount of nitrites added to meat products in the past, had caused deaths due to intoxication, in early decades of 20th century in Germany (Honikel, 2008).

The main adverse effect related to acute toxicity of nitrites is methaemoglobinaemia, manifested as cyanosis, usually as a consequence of excessive nitrites exposure. Infants under 3 months of age are more susceptible than older children and adults due to nitrites inhibiting the transport of oxygen to hemoglobin (WHO, 2007). A case of acute human intoxication to nitrites in a 40 year-old mother and her 9 years old child was recently described in Italy, after the consumption of turkey contaminated with high nitrites levels ranging from 6,000 to 10,000 mg kg<sup>-1</sup> (Matteucci et al., 2008).

The long-term toxicity of nitrates and nitrites is associated with their potential to form carcinogenic NOCs in the food matrix as well as in the human body. NOCs are formed when various nitrosating agents originating from nitrites (Andre et al., 2010; Honokel, 2008) interact with the amide compounds either in food or in the stomach. The NOCs can be divided into two classes: the first class is nitrosoamines (NA) and the second class is nitrosoamide-type compounds such as N-nitrosoureas, N-nitrosocarbamates and N-nitrosoguanidines. NOCs of both classes differ considerably in their chemical formation and biological effectiveness (Reinik et al. 2005).

The NA commonly found in meat products are the volatile substances N-nitrosodimethylamine (NDMA), -piperidine (NPIP), -pyrrolidine (NPYR), -diethylamine (NDEA). NDMA and NDEA are usually found in some thermally cured products (Reinik et al. 2005).

NA are formed by a nitrosylation of the secondary amines. Also primary amines can be nitrosated, but these products are not stable. Nitrosation of tertiary amines is not possible (Andrée et al., 2010). The



process of nitrosation is very complicated and depends on many factors such as pH, the type of the secondary amine and temperature (Ward and Coates, 1987).

NA formation is favored under the following conditions (Andrée et al., 2010):

- 1) Secondary amines should be present. In fresh meat, the secondary amines are either not present or are found at very low amounts. Most amines in meat are primary amines derived from  $\alpha$ -amino acids. Potential precursors of secondary amines like creatine and creatinine, the free amino acids proline and hydroxyproline, as well as some decarboxylation products can lead to formation of secondary amines during fermentation and storage of meat products
- 2) The pH must be low (less than 6.55) to form nitrosating agents. The low pH is only important for fermented sausages.
- 3) Thermal processing at high temperatures, usually above 130 °C (formation of NPYR), or long storage at room temperature (formation of NDMA, NPYR). Grilling, roasting or frying of meat products could form NA.

Several epidemiological studies have suggested NA as the etiological agents of various types of cancer in humans (Eichholzer and Gutzwiller 1998; Pegg and Shahidi 2000). Several NOCs tested in different animals proved to cause cancer (Tricker and Preussmann, 1991).

The International Agency for Research on Cancer (IARC, 1987) classified NDMA in group 2A (*Probably carcinogenic to humans*) and NPIP, NPYR in group 2B (*Possibly carcinogenic to humans*). Recently, the IARC concluded that ingested nitrates or nitrites are probable carcinogen to humans under conditions favoring the endogenous nitrosation (IARC, 2010).

Studies showed that high doses of nitrites have a toxic effect on reproduction (Fan and Steinberg 1996; Manassaram et al. 2006). Nitrites themselves or the nitrites degradation compounds have been found to be mutagenic, since they can increase the number of micronucleated polychromatic erythrocytes in vivo in mice (Luca et al. 1987).

Reviewing the toxicological effects of nitrates and nitrites an acceptable daily intake (ADI) for nitrates

of 3.7 mg kg<sup>-1</sup> bw has been established by FAO/WHO (2003). The ADI for nitrites is 0.07 mg kg<sup>-1</sup> bw according to FAO/WHO (2003) and 0.06 mg kg<sup>-1</sup> bw according to the SCF (1995) of European Commission. In 2008, the above nitrates and nitrites ADI levels were also endorsed by EFSA (2008).

Recent studies indicated a beneficial effect to health of the nitrates and nitrites in foods, when consumed at certain low doses. According to these works, the nitrites are associated with lower blood pressure and better cardiovascular function as well as play an important role in vasoregulation (Lundberg et al. 2006, 2011; Lundberg and Weitzberg, 2009). In view of recent data, potential risks from nitrates and nitrites intake may also need to be reconsidered (Lundberg and Weitzberg, 2009).

## LEGISLATION

Nitrates (potassium nitrate, E251; sodium nitrate, E252) and nitrites (potassium nitrite, E249; sodium nitrite, E250) are listed as official food additives in the corresponding Commission Regulation (EU) No. 1129/2011. In order to protect consumers and keep the quality characteristics of cured meat products, certain legal limits were set for nitrites and nitrates by various countries. European Union set also limits for nitrates and nitrites for a great variety of meat products manufactured and traded throughout the European Union countries, by regulating either the ingoing or the residual amounts of these salts in the products [Commission Regulation (EU) No. 601/2014]. However, objections on the limits of residual levels of nitrites were also raised by scientists since the measurement of the residual levels of nitrites in the final product may be of a limited value (EFSA, 2003).

The legal limits established in Regulation (EU) No. 601/2014 for nitrates and nitrites in meat products in EU are summarized in Table 1.

## NITRATES AND NITRITES CONTENT IN MEAT PRODUCTS-

Due to growing consumer concern over the potential negative health effects of consumed foods with nitrates –nitrites additives, several surveillance

**Table 1.** Nitrates and nitrites legal limits reported in Regulation (EC) No. 601/2014.

MEAT PRODUCTS	LEGAL LIMIT (mg/l or mg/kg as appropriate)		Limit application
	NITRATES <sup>1</sup>	NITRITES <sup>2</sup>	
Non-heat-treated processed meat	150	150	Maximum amount that may be added during manufacturing.
Heat-treated processed meat Except sterilised meat products ( $F_0 > 3.00$ ) <sup>3</sup>	-	150	Maximum amount that may be added during manufacturing.
Only sterilised meat products ( $F_0 > 3.00$ ) <sup>3</sup>	-	100	Maximum amount that may be added during manufacturing.
Traditionally cured meat products with specific provisions concerning nitrites and nitrates	10–300	50–180	Depends on product: Maximum added amount or maximum residual amount, residue level at the end the production process

<sup>1</sup> E251 (sodium nitrate), E252 (potassium nitrite).

<sup>2</sup> E249 (potassium nitrite), E250 (sodium nitrite).

When labelled “for food use”, nitrites may be sold only in a mixture with salt or a salt substitute.

<sup>3</sup> Fo-value 3 is equivalent to 3 minutes heating at 121 °C for *C. botulinum*.

programs were conducted for the estimation of the nitrates and nitrites levels in cured meat products sold in the markets of several EU countries as well as in other countries of the world.

EFSA (2003) published a survey with data from official monitoring program of residual nitrites level in cured meat products sold in certain countries of EU (France, Belgium, Ireland, and Germany) in the years 1990 – 2003. The summarized data from this survey (EFSA, 2003) are as follows. In France, 3,112 samples of raw dried, cooked and cured meat products were analyzed in 1995 and 2002. The levels of residual  $\text{NaNO}_2$  of cured meat products ranged from 0 to 9 mg  $\text{kg}^{-1}$  in 59% and 74% of the samples in the years 1995 and 2002, respectively. In Belgium,

the levels of residual nitrites were below 20 mg  $\text{kg}^{-1}$  in 76% of 75 cured meat samples in the year 2002, while the same levels of residual nitrites were in 71% of 24 cured meat samples in the year 2003. In Ireland, 147 samples of bacon samples were analyzed in 2001 and 2002. The levels of residual  $\text{NaNO}_2$  were 0-20 mg  $\text{kg}^{-1}$ , 20–29 mg  $\text{kg}^{-1}$ , 30-39 mg  $\text{kg}^{-1}$  and 40-49 mg  $\text{kg}^{-1}$  in 36%, 20%, 12% and 7% of the bacon samples, respectively. In Germany, 116 samples of cured meat were analyzed for residual levels of nitrites in the years 2001 and 2002. The levels of residual  $\text{NaNO}_2$  in 85% of the samples were below 20 mg  $\text{kg}^{-1}$ . According to this survey (EFSA, 2003), the vast majority of cured meat samples complied with the current regulation on the levels of residual nitrites in EU.

In United Kingdom, 200 samples of cured meat products were examined for residual nitrites levels in a survey conducted in 1997 (MAFF, 1998). The residual nitrites levels were 0.2 - 123 mg kg<sup>-1</sup> for bacon and 0.2 - 170 mg kg<sup>-1</sup> for other meat products (MAFF, 1998). The nitrites level of examined cured meat products of UK was comparable to that of imported products.

In Finland, according to a survey conducted in 1994 (Penttila, 1995), the mean concentrations of nitrites and nitrates in cooked sausages were 48 mg kg<sup>-1</sup> (range 0–124 mg kg<sup>-1</sup>) and 32 mg kg<sup>-1</sup> (1.4–53 mg kg<sup>-1</sup>), respectively.

In Denmark, Leth et al. (2008) reported the content of nitrites and nitrates in cured meat products from data obtained from the official Danish food monitoring program for food additives in the years 1995-2006. The average level of nitrites was low and in accordance with the Danish legislation (60 mg kg<sup>-1</sup> for most products and up to 150 mg kg<sup>-1</sup> for certain products). It also seemed that the nitrites levels in cured meat products were fairly stable over the years, with an exception in 2002.

In France, Menard et al. (2008) reviewed existing data of 13, 657 concentration values of nitrates and nitrites which were measured in food representing 138 and 109 food items, respectively, during official monitoring programs between 2000 – 2006. They concluded that 8% of the meat products and 6% of the industrial meat products exceeded the nitrates maximum residual level of EU. They also found that 0.4% of industrial meat products and 0.2% of meat products were not in accordance with the nitrites European maximum residual level.

In Russia, Zhukova et al. (1999) examined the nitrites level in 186 meat products during refrigerated storage for 30, 60 and 90 days. The nitrites levels were in the range of 0.2 – 9.1 mg kg<sup>-1</sup>, which were in accordance with Russian legislation.

In Greece, 30 samples of pastirma (a traditional cured meat product) were examined with the concentrations ranging from 0.85 to 189.65 mg kg<sup>-1</sup> for nitrites and from 2.66 to 639.67 mg kg<sup>-1</sup> for nitrates (Tyrpenou et al., 2000).

In Estonia, Reinik et al. (2005) examined the nitrates

and nitrites content of 189 meat products (cooked sausages, smoked sausages and ham) in 2000 – 2004. All the samples showed nitrates and nitrites levels within the Estonian legislation set to 100 and 250 mg kg<sup>-1</sup> for the nitrites and nitrates levels, respectively. In 2000 the mean nitrites concentration in cooked sausages was 38 mg kg<sup>-1</sup>, but in 2004 it was 30 mg kg<sup>-1</sup>. A similar tendency was registered for smoked sausages and ham products.

In Canada, Sen and Baddoo (1997) studied the data from official monitoring programs concerning nitrites levels in various meat products in 25 years time (1972-1997). They reported the following mean nitrites levels in cured meat products: 28 ppm (0–252 ppm; *n* = 197) in 1972; 44 ppm (0–275 ppm; *n* = 659) in 1983–1985; 31 ppm (1–145 ppm; *n* = 76) in 1993–1995; and 28 ppm (4–68 ppm; *n* = 35) in 1996. The mean nitrites contents of sausages were 34 and 26 mg kg<sup>-1</sup> in 1985 and 1996, respectively. The highest levels were detected in uncooked cured meat products (pastrami, smoked beef, spiced beef). At the time of the survey the maximum permissible nitrites level in cured meat products under the Canadian legislation was 200 mg kg<sup>-1</sup>.

In USA, Cassens et al. (1997) examined 164 samples of cured meat (bacon, bologna, sliced ham, and wieners) obtained from retail market. The estimated levels of residual nitrites were in the range of 0–48 mg kg<sup>-1</sup> (mean values 10 mg kg<sup>-1</sup>). Keeton et al. (2009) reported residual nitrites levels of 7 ppm in cured meat products (hot dogs, bacon and hams) from five cities of USA. The findings of Keeton et al. (2009) are consistent with those from an earlier nationwide survey conducted by Buege et al. (2002). In contrast, the residual levels of nitrites reported in an early study of National Academy Science (NAS, 1981) were higher with values ranging from 10–31 ppm in hot dogs, 12–42 ppm in bacon, and 16–37 ppm in hams. Results from both studies (Buege et al. 2002; Keeton et al. 2009) showed an approximately 80% reduction in nitrites levels as compared to those found by a similar survey conducted in 1975 (White, 1975). In this early survey, White (1975) examined the residual nitrites in cured meat products and found values of 0

to 272 mg kg<sup>-1</sup> (mean 52.5 mg kg<sup>-1</sup>). In a recent study, Nunez De Gonzalez et al. (2012) estimated the residual nitrites in 470 cured meat products representing six major categories, which were taken from retail outlets in five major metropolitan cities across the United States. Nitrites concentrations were similar to those previously reported by Cassens et al. (1997). Thus, in most recent studies the residual levels of nitrites in cured meat samples were reduced as compared to the level in the early studies in USA.

In Belgium, De Mey et al. (2014) examined the nitrates and residual nitrites levels in 101 cured meat samples. They found mean values of nitrates and residual nitrites lower than 20 mg kg<sup>-1</sup>.

In Iran, Rezaei et al. (2013) estimated the nitrites levels in hamburgers sold in Arak city, in the centre of Iran, in 2011. The residual nitrites in the samples were in the range of 30-100 mg kg<sup>-1</sup>. They concluded that the high residual levels of nitrites were due to unfavorable production conditions and poor sodium nitrite standards at hamburger factories.

In Australia, Hsu et al. (2009) conducted a survey for the residual nitrites in various food products. In cured and fresh meat samples, nitrates content ranged from 3.7 to 139.5 mg kg<sup>-1</sup>, and nitrites content ranged from 3.7 to 86.7 mg kg<sup>-1</sup>. These values were below the regulatory limits set by food standards Australia and New Zealand (FSANZ).

Although it is difficult to compare data for nitrates or nitrites levels in cured meat in surveys conducted in different years (due to sampling methods, analytical

methods etc), a marked decline of the nitrites content of meat products is indicated over the last 20 years. These lower levels could be attributed to changing production technologies, decreased levels of ingoing nitrites and the wider use of cure accelerators such as sodium or potassium ascorbate and erythorbate. Additional reasons could also be related to the public awareness of the potential adverse health effects of nitrites/nitrates additives in meat products as well as the legislation changes imposed for cured meat products (Cassen, 1997; Sindelar and Milkowski, 2012).

### CONCLUSIONS

Nitrites and nitrates are two important additives in meat industry because of their beneficial effect on the quality and microbiological safety in meat products. On the other hand, the major concern of nitrates/nitrites in meat products is related to the potential of nitrites to form carcinogenic N-nitroso compounds. Surveys from various countries indicate an important decline in the nitrites content in meat products over the last 20 years. According to recent surveys, the levels of residual nitrites found in meat products cannot endanger consumers' health. Nitrites at low doses proved to be beneficial to human health, since it is also associated with lower blood pressure, better cardiovascular function and plays an important role in vasoregulation, as indicated in recent works.

### CONFLICT OF INTEREST

None to declare. ■

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