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Nitrate, Nitrite and N-nitrosamine in Meat Products

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Abstract

Food preservative additives are natural or synthetic substances that prevent microbial development, enzyme activity, and oxidation from causing food degradation. According to EU Regulation No. 1129/2011/EC 2011, nitrite (E249, E250), nitrate (E251, E252), and nitrite are allowed food additives in the European Union and are widely used to preserve meat. It has a great deal of technological utility and helps produce cured meats' distinctive pink colour, antioxidative activity, and flavour. It also inhibits the growth of bacteria that cause food spoilage, particularly Clostridium botulinum, which creates the potentially lethal toxin botulin. The most obvious and recognisable indication of nitrite toxicity in humans is methemoglobin production. In addition, certain amines in food may interact with nitrites to generate N-nitrosocompounds, several of which are known carcinogens. There is rising interest in using natural antimicrobials as food preservatives due to the negative short- and/or long-term effects of consuming chemical food preservatives over time, since synthetic preservatives can cause health issues, replacing them with natural preservatives is much better for humans and the environment. This review is discussing the possibility of using plant extracts, plant smoke presented as liquid smoke and the plant itself in preserving meat products resulting in clean label products. These technical processes may be well embraced.

Keywords:

Nitrate, Nitrite, N-ntrosamine, Natural alternatives, Meat products.

INTRODUCTION

Food preserving ingredients are chemicals, either natural or manmade, that stop oxidation, microbial growth, and enzyme activity from causing food to degrade. In the past, artificial food additives were more frequently used. Yet, consumers have not usually welcomed synthetic chemicals in recent years due to claims that they have harmful health impacts (Gokoglu, 2019). Despite their enormous technological utility, nitrites also produce cured meats' particular flavour, contribute to the creation of a distinctive pink color, and have antioxidant properties, and inhibit the growth of food spoilage bacteria, particularly Clostridium botulinum, which produces the potentially fatal toxin botulin (Deda et al., 2007). Nitrite and nitrate are the two preservatives used in meat processing most frequently (Farag & Abd-el-fatah, 2011). Nitrate (E251, E252) and nitrite (E249, E250) are extensively used food additives in the European Union that are permitted pursuant to EU Regulation No. 1129/2011/EC 2011 (Merino et al., 2016). The most obvious and recognisable sign of nitrite poisoning in humans is methemoglobin production. Additionally, when nitrites interact with particular amines contained in food, Nnitrosocompounds can be produced, several of which are known carcinogens. Since the rate of nitrosamine synthesis is proportional to the square of nitrite concentration, lowering the nitrite concentration has a greater impact on the amount of nitrosamines produced in meat products. Due to the potential health danger posed by nitrites, there is a significant lot of interest in the creation of natural food colourants, which are believed to be healthy and of outstanding quality (Deda et al., 2007). As a result, consumers' preference for natural additives is growing by the day. Natural preservatives can be found in plants, microbes, fungi, animals, and algae (Gokoglu, 2019). Depending on the shape, composition, and concentration of the organism, natural herbs and spices have different preservation qualities. Herbs and spices might contain volatile compounds. Preservatives are made from these chemicals, which are extracted using a variety of techniques and contain substances that serve as defensive mechanisms in plants (Gokoglu, 2019). The primary antimicrobial compounds found in plants and their extracts and essential oils (EOs) are phenolic compounds such as cinnamic aldehyde from cinnamon, thymol from thyme and oregano, eugenol from clove and cinnamon, carvacrol from oregano, and anethole from anise. New antibacterial substances are frequently identified from bacteria, fungi, herbs and spices are considered alternative sources (Hintz *et al.*, 2015).

Nitrate and Nitrite in meat and meat products

Meat curing's precise historical origin is unclear, although it is thought to have been discovered by mistake (Sindelar, 2006). Ancient civilizations either mined salt directly from the earth or evaporated water from brine pools or ocean to obtain it from crystalline formations. Natural impurities like sodium or potassium nitrate (also known as saltpetre or "niter") were consequently commonly present and directly influenced the curing reaction and preservation process (Keeton, 2011). According to Sumerian Tablets written in cuneiform script, several dried and cured meats were available by 2000 BC. By 770 BC, the Zhou dynasty had developed traditional meat processing in eastern and southwestern China (Chen et al., 2015). Nitrate concentrations might reach 10,000 parts per million when Europeans layered nitrogenrich soil with lime and water to create saltpetre in the 1500s (Barnum, 2003). Nitrosation of amines was first discovered in the 1840s, and studies proceeded to find other nitrosated/nitrosylated compounds (Williams, 2004). With more salt being used to preserve meat, people began to choose salts that generated a pink colour and distinct flavour over a less appealing grey tint. (Mulvey et al., 2010). As sugar became more accessible during the Middle Ages, curing advanced, and saltpetre use in meat curing became a common practise. Sweet cures were also developed. By the end of the nineteenth century, numerous curing techniques (dry cures, wet cures, and variations of both) had been created (Mulvey et al., 2010). Depending on whether the cure is blended with the mince or added on the surface of the meat dry, cured meat products are classified as either dry-cured or wetor pickle-cured (Toldra', 2017). Early huntergatherers needed to improve meat and food storage to increase their harvest and expand their food supply by delaying spoilage. Early humans preserved food by freezing, salting, and drying it, and the local environment affected the preservation technique (Sullivan, 2011). Curing meat and poultry are one of the oldest methods of food preservation that has evolved (Sindelar and Milkowski, 2012). Thousands of years ago, salting meat to keep it fresh without refrigeration was invented. Furthermore, the effectiveness of drying in meat preservation has been empirically accepted since ancient times (Chen et al., 2015). The technique of applying salt (sodium chloride) to fresh meat pieces in order to draw out moisture and lower tissue water activity in order to prevent deterioration is known as meat curing (Keeton, 2011). The application of a curing salt or cure containing sodium chloride, nitrate, and/or nitrite, which aids in the development of a cured pink or reddish colour and flavour in the meat, is the traditional definition of curing. The definition of "cured meat" varies based on the type of product and the place of origin, despite the fact that term is frequently used and refers to a variety of meat products worldwide. (Toldra', 2017).

The functions of nitrate and nitrite in meat products

The appearance, taste, purity, and consistency of cured meats are improved by nitrite (and its predecessor, nitrate). For example, the distinctive reddish-pink color and flavor of cured meats are due to nitrite. In addition, nitrite improves meat safety by inhibiting the growth of microorganisms, especially *Clostridium botulinum*, inhibiting lipid peroxidation, nitrite retains and can even enhance the flavor by preventing rancidity (Bedale *et al.*, 2016).

1. Color development

The formation of an attractive reddish-pink cured color is the earliest and most noticeable consequence of nitrite (Parthasarathy and Bryan, 2012). The color of meat is essential because it influences the consumer's palatability and quality. Because of this, it is necessary to add nitrate or nitrite throughout the manufacturing process to guarantee that the beef product develops the right cured colour (Grossi *et al.*, 2014). When nitrite is added to meat, a series of procedures are carried out before it combines with the pigment (Usinger, 2016). Myoglobin is the pigment that gives meat its

color. The amount of myoglobin present and the pigment's chemical condition affect the colour of meat. There are three main types of myoglobin depending on whether the pigment has been reduced or oxidised. Myoglobin is a purple-red color in raw meat, whereas oxymyoglobin is a brilliant red color and metmyoglobin is a brown color. Myoglobin can be oxygenated to produce oxymyoglobin, and myoglobin can be oxidized to produce metmyoglobin (Pegg and Shahidi, 2000). Cured beef contains nitrosylmyoglobin, which gives uncooked cured food its red colour. Nitric oxide and myoglobin interact to produce it. Sodium nitrite, which is in equilibrium with undissociated nitrous acid in the solution, is the most frequent source of nitric oxide. Nitrous acid transforms into nitric oxide when exposed to mildly acidic circumstances. Myoglobin is quickly transformed into metmyoglobin, which nitrite, an oxidising agent. subsequently reduces nitrosvl metmyoglobin. With input nitrite at 100-150 ppm, myoglobin conversion to the nitrosyl form can vary between 35 and 75% in various meat samples (Ranken, 2000). When heated, nitrosyl myoglobin is denatured to pink nitrosyl myochromogen, commonly known as cooked cured meat pigment (CCMP). Rather than giving a color, nitrite fixes the pink color of cured items (Pegg and Shahidi, 2000). The pigment that gives cured meat its colour is not fixed by the highly reactive ion nitrite on its own; rather, nitrosylating agents produce and transport nitric oxide to the pigment, fixing the colour. Nitric oxide production from nitrite is needed to produce cured colour (Sindelar and Milkowski, 2012). Nitric oxide and muscle myoglobin combine to form nitric oxide metmyoglobin, which is what gives cured meats their brown colour before being heated, in the presence of endogenous or external reductants. Myoglobin's protein component is denatured during cooking, releasing the mono-nitrosated heme structure from its covalent bond and creating nitrosylhemochromogen (Usinger. 2016). According to Sebranek and Bacus (2007), residual nitrite levels of 45-119 ppm are adequate to produce the hues of cured meat. The levels of nitrite needed for colour formation and stability in bacon should be between 10 and 20 ppm in heated bacon at the time of cooking, according to Ranken (2000). Bacon with little or no nitrite at frying may

turn grey after cooking. Nitric oxide reacts with myoglobin to produce nitrosylmyoglobin, which gives cured meat its ideal red colour at an initial concentration of roughly 20 mg/kg of NaNO₂ (Hsu, 2009). As a result, most goods only need 40 to 50 ppm of incoming nitrite to produce a cured colour (Usinger, 2016). Color stability over time was thought to depend on residual nitrite by Walsh et al., (1998). Mulvey et al., (2010) stated that the quantity of myoglobin in the tissue determines the amount of nitrite required for color fixing, which is influenced by species, sex, age, muscle type, age, and diet. The initial amount of sodium nitrite used into meat for curing (typically 156 ppm) rapidly decreases due to reactions during formulation and heating, leaving residual nitrite in the product at a much lower concentration, which continues to diminish throughout storage (Usinger, 2016).

2. Flavor development

The second quality characteristic resulting from nitrite in meat curing is the development of the cure flavour. Sadly, there is little particular literature on the chemistry of cured beef flavour (Pegg and Shahidi, 2000). Though there are many hypotheses on how nitrite forms flavours, the mechanism fundamental is still unknown (Villaverde et al., 2014b). According to some theories, the antioxidant properties of nitrite, which stop lipid oxidation, are what cause flavour to be formed (Usinger, 2016). Compared to products containing nitrite, the addition of alternative antioxidants did not result in similar flavor development, indicating that the flavor created is more than just an antioxidant impact (Sebranek and Bacus, 2007). Additionally, sensory studies have revealed that, rather than only delaying lipid oxidation, cured flavour is produced by complex chemical combinations working together without the use of rancid odours (Sindelar and Milkowski, 2012). The bacon flavour and the amount of nitrite in the brine used to cure Wiltshire bacon were discovered to be linearly correlated by Tichivangana et al., 1984. Similar investigations discovered that increasing concentrations are associated with increasing product panel approval scores (Pierson and Smoot, 1982). According to Feiner (2007), the flavor of cured meat is created by nitrite components reacting with sulfuric material found in animal

muscle. Thiobarbituric acid, a measure of rancidity, can drop by up to 64% in beef, pig, and chicken when nitrite levels are as low as 50 ppm, which has been shown to have an antioxidant effect (Sebranek and Bacus, 2007). Moreover, nitrite combined with salt at a rate of 50 mg/kg of NaNO₂ gives cured meat its flavor (Hsu, 2009).

3. Antioxidant activity

It is well-known that meats that have been cured have greater oxidative stability. As a result, cured meats can undergo a variety of processes that lengthen their shelf life. Lipid oxidation can start in a variety of ways, and once it does, free radical interactions cause it to increase exponentially (Wong, 1989). Nitric oxide reacts swiftly with oxygen and other reactive oxygen species, sequestering them (Ford and Lorkovic, 2002). Nitric oxide, a free radical, can aid in halting lipid auto-oxidation (Pegg and Shahidi, Bergamaschi and Pizza (2011) found that nitric oxide binds free iron and stabilizes heme iron, minimizing lipid oxidation by restricting the iron's pro-oxidant activity. Lipid oxidation targets unsaturated fatty acids, and nitrosation of double bonds may reduce lipid oxidation. It has been demonstrated that adding 50 ppm sodium nitrite reduces lipid oxidation products by about 65 % (Sullivan, 2011). The antioxidant capabilities of nitrite are governed by the exact mechanisms that generate cured color and flavor (Sebranek, 2009). The creation of nitrosylhemochromogen and the generation of nitric oxide are two of nitrite's two antioxidant functions. The immobilization of the iron complex by nitrosylhemochromogen reduces catalytic activity and prevents the beginning of lipid oxidation. The second antioxidant activity is attributed to nitric oxide, which functions as a free radical acceptor and stops the free radical chain reaction to prevent lipid oxidation (Usinger, 2016). By bonding together and maintaining the heme iron in the meat's pigment, nitric oxide can minimize the quantity of free iron released during cooking (Parthasarathy and Bryan, 2012). By limiting the development of warmed-over flavour (WOF) in cooked cured products and by ensuring long-term taste stability, preventing lipid oxidation enhances cured flavour (MacDonald et al., 1980; Sebranek and Bacus, 2007). In addition, cured meats can maintain a superior product quality through distribution and storage by preventing the development of oxidative rancidity (Sindelar and Milkowski, 2012). Because nitrite has these beneficial antioxidant qualities, the USDA prohibits synthetic antioxidants in most products that already contain nitrite. However, It is acceptable to include synthetic antioxidants with nitrate for dry cures because nitrate curing in drycured hams or fermented sausages is a lengthy process (Sindelar and Milkowski, 2012), and further fat oxidation prevention is needed (Usinger, 2016).

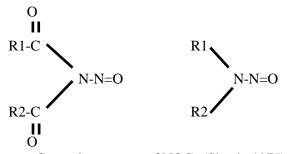
4. Antimicrobial activity

Aside from flavoring and coloring meat, Nitrite has the peculiar ability to prevent the growth of Clostridium botulinum spores. This pathogen has long been the focus of research into nitrite's antimicrobial properties (Sebranek and Bacus, 2007). Listeria monocytogenes, which can thrive in high salt and cold temperatures, has recently grown to be a concerning source in ready-to-eat meats. (Swaminathan, 2001). Listeria monocytogenes and other microorganisms can be controlled by nitrite, despite the fact that it is a more powerful anaerobic bacterium inhibitor (Sebranek and Bacus, 2007). In addition to nitrite, numerous other nitric oxide donors have been studied and shown to have antibacterial properties comparable to those of cured meats (Cammack et al., 1999). Nitrite acts as a bacteriostatic and bacteriocidal agent, despite its exact mechanism being unknown. The processes involved in nitrite's conversion to nitric oxide are thought to be responsible for its antibacterial properties (Sindelar and Milkowski, 2012). Nitrite is believed to have a variety of effects on bacteria, including the restriction of oxygen uptake, the disruption of the proton gradient, and the inhibition of metabolic enzymes (Yarbrough et al., 1980). Similar to Roussin's salts, synthetic iron-sulfur complexes and nitric oxide react to form these colors (Harrop et al., 2008). The nitrosylation of iron-sulfur complexes inactivates ferredoxin and pyruvate: ferredoxin oxidoreductase (Rahman, Cytochrome-c, a heme-centered protein that moves electrons from complex III to IV, is inhibited by the presence of nitrite (Walters and Taylor, 1964). The binding of nitric oxide to iron regulates and restricts the availability of iron, which is required for enzyme function and bacterial metabolism and growth (Tompkin, 2005). Because of their high reactivity, iron sulfur complexes and the heme iron centres of enzymes are usually targets of nitrite (Cui et al., 1992). Cysteine is a component of enzymatic processes and signalling many pathways, and it is likely that nitrite will also block this compound. When membrane sulfhydryl groups are nitrosated, the signalling system and the cell's ability to react to its environment are both compromised (Morris et al., 1984; Gaston, 1999). S-nitrosation in the mitochondria blocks Complex I, the entrance point of the electron transport chain (Shiva, 2010). Pyruvate buildup in bacteria living in nitrite environments shows that the electron transport chain, a more effective pathway for ATP generation, has been deactivated (Woods et al., 1981). Others discovered sulfhydryl group nitrosation caused reductions in bacterial glycolytic enzymes (O'Leary and Solberg, 1976). These complexes are present in some species of Pediococcus. Listeria. Escherichia Staphylococcus aureus, and Clostridia, and they explain why nitrite inhibits these microorganisms (Rahman, 2007). Most lactic acid-producing bacteria, including most species of Salmonella, do not have these complexes and are not inhibited by nitrite (Jay, 2000). The microbiological functions of nitrite in inhibiting C. botulinum include enhancing heat-induced spore destruction, increasing spore germination during thermal processing and then destroying the germinated spores with heat, preventing spore germination and and reacting with some outgrowth, component(s) to form a more inhibiting compound(s) (Usinger, 2016). Antimicrobial action necessitates a higher concentration of nitrite than other cured meat qualities. Thus, it is estimated that in commercial settings, a residual NaNO2 concentration of at least 100 mg/kg is required for protection (Hsu, 2009).

N-nitrosamine in meat and meat products

Nitrosamines are a class of carcinogens that are acutely toxic, mutagenic, and transplacental. They have been discovered in meals, particularly those processed with nitrate or nitrite, in a range of alcoholic beverages, and as occupational pollutants in the workplace. For example, rubber, tanning, and metalworking are all industries that use rubber.

Likewise, cosmetics and hygiene, nicotine and cigarette smoke, certain types of drugs, pesticides, cutting oils, and other industrial chemicals are all potential sources of exposure (Luque-Pérez and Valcárcel, 2001). Throughout the past fifty years, N-nitroso compounds (NOCs) have attracted a lot of attention. According to data, there is a higher risk of gastrointestinal cancer when dietary nitrate and nitrite intake grows. Both anions are frequently present in food, and because of their reactivity and metabolites, they may have an effect on a number of physiological systems. (Hsu, 2009). Ingested nitrite can attach to various precursors or nitrosatable amines to create N-nitroso compounds (NOCs), which are potent carcinogens (IARC, 1987). They are carcinogenic because they produce potent electrophilic alkylating agents. The bases in the DNA of the target tissue will then change as a result of the electrophiles' reaction with it, starting the process of carcinogenesis. (Archer, 1989). Nnitrosamine and N-nitrosamide are two important classes of NOCs. The former has to undergo metabolic modification via cytochrome P450dependent hydroxylation to produce the alkylating agent -hydroxynitrosamine (Tricker Preussmann, 1991). N-nitrosamide, on the other hand, decomposes into an alkylating agent, is chemically reactive, and is unstable physiological pH (Shank, 1975).



General structures of NOCs (Shank, 1975)

Despite the fact that there are hundreds of NOCs, only a small number are found in the environment, industrial settings, and food that people consume. The most prevalent NOC is nitrosamine, which is stable and has an indirect action. However, in animal trials, nitrosamines were found to cause tumors in various organs, including the liver, lung, kidney, bladder, pancreas, esophagus, and tongue. On the other hand,

nitrosamides, such as alkylnitrosoureas and alkylnitrosocarbamates, are unstable and have a direct effect and can induce cancer in animals' stomachs, gastrointestinal tracts, and bones (Lijinsky, 1999). NOCs were categorised by Pourazrang et al., (2002) into six groups: there are six different types of N-nitrosamine: volatile Nnitrosamine, non-volatile N-nitrosamine, nitrosamide, N-nitrosated glycosylamines and amadori compounds, Nnitrosated heterocyclic carboxylic acids, and byproducts of the reaction between amino sugar and nitrite. A wide range of foods, such as cured pork, contain varying carcinogenic volatile N-nitrosamines, nitrites, and nitrate (Hassan and Ali, 2010). NOC levels and digestion pH both increased with ageing and was positively correlated with both an increase in nitrite concentration **NOCs** concentration. Furthermore, as NOCs and nitrite levels grew, the number of nitrate reductase-positive bacteria increased (Reed et al., 1981). Because of the rise in pH, microorganisms can survive in the stomach and turn nitrate into nitrite. As people age, levels of N-nitrosamine may rise due to increasing nitrosation because nitrite is more persistent at higher pH levels. (Hsu, 2009).

Formation of Nitrosamines

In cured meat products, N-nitrosamines are generated by the interaction of a secondary amine with nitrite at high temperatures and a low pH. Only secondary amines can react, thus they are required. It's crucial to keep in mind that primary amines quickly break down into alcohol and nitrogen while tertiary amines don't produce nitrite. Nitrous anhydride (N2O3) is created in acidic conditions from nitrite (NO⁻²), which is then converted to NO and NO2. An unprotonated secondary amine can interact with NO-, which is produced in the presence of a transition metal ion like Fe⁺³, to create N-nitrosamines through a nucleophilic substitution process. (Pegg and Honikel, 2015).

$$2NO_2^- + 2H^+ \longrightarrow N_2O_3 + H_2O$$

$$N_2O_3 \longrightarrow NO + NO_2$$

$$NO + Fe^{+3} \longrightarrow NO^+ + Fe^{+2}$$

$$R_2NH + NO^+ \longrightarrow R_2-N-N=O + H^+$$
Formation of N-nitrosamine (Pegg and Honikel, 2015).

Of course, encouraging factors such as residual nitrite, low pH, and degree of heating (e.g., frying, grilling, baking) affect the quantity of nitrosamines produced (Herrmann et al., 2015a). The presence of sulfhydryl compounds, certain phenols, and tannins in the meat product are some of the elements that regulate the nitrosation processes, all of which to varied degrees limit the creation of nitrosamines, with ascorbic acid being the most significant (Pegg and Shahidi, 2000). In reality, adding ascorbic acid, ascorbate, or erythorbate to curing formulations at a concentration of ~500 mg/kg has long been a typical practice in industry since it helps to prevent the production of nitrosamines. Even though nitrite is reduced to NO and ascorbate is oxidized to dehydroascorbate, there have been concerted efforts to eliminate residual nitrite levels in cured meat products (Toldrá, 2017). There are both volatile and nonvolatile nitrosamines. The four most frequently found and reported volatile nitrosamines in meat products are N-nitrosodimethylamine (NDMA), Nnitrosopiperidine (NPIP), and N-nitrosopyrrolidine (NPYR). Although very few nitrosamines have been discovered in raw bacon before it has been fried, NPYR and NDMA are typically identified in bacon after it has been fried. The amount of volatile nitrosamines in 70 Danish and 20 Belgian items was recently studied, and the mean levels of each nitrosamine amount were frequently reported to be less than 0.8 mg/kg (Herrmann et al., 2015b). Nonvolatile nitrosamines (NVNA) are principally N-nitrosamino acids, found in substantially larger concentrations in processed meat products than volatile nitrosamines (Crews, 2010). Individual NVNA levels in products from the Danish and Belgian markets, however, averaged around 118 mg/kg, with greater levels seen in particular types of hams and sausages (Herrmann et al., 2014). The often recognised **NVNAs** two most are Nitrosothiazolidine-4-carboxylic acid (NTCA) and nitroso-2-methylthiazolidine-4-carboxylic acid (Herrmann et al., 2015b).

Nitrate, Nitrite and human health

Nitrite (and its predecessor, nitrate) in food has an illogical association with consumers. Despite its long history of use, nitrite was nearly prohibited from food use in the 1970s due to health concerns about the formation of carcinogenic nitrosamine

(Bedale al., 2016). Excessive nitrite etconsumption can result in tissue poisoning, respiratory center paralysis, and other hypoxiarelated symptoms. Because nitrite reduces the oxygen-carrying capabilities of low-iron hemoglobin in human blood by oxidizing it to methemoglobin, it can cause asphyxia and death in severe cases. Meanwhile, nitrosamines are formed when protein-derived amines in meat products mix with nitrosating agents, and they have a high carcinogenic effect in humans, particularly in gastric cancer. High nitrite intake can disrupt iodine metabolism and reduce iodine uptake by the thyroid, resulting in thyroid gland hypertrophy (Huang et al., 2020).

Nitrate and methemoglobinemia

Methemoglobinaemia, or the binding of nitrite transformation products to hemoglobin with the resulting decrease of oxygen transport capacity, is a well-known health consequence of nitrite in humans (Merino et al., 2016). The development of infantile methemoglobinemia, a disease in which the iron molecule in haemoglobin is oxidised from ferrous (Fe⁺²) to ferric (Fe⁺³), affecting red blood cell oxygen transfer to tissues, was linked in the 1940s to nitrate ingestion. Although it has recently been questioned whether this effect is caused by bacterial contamination of the nitrate source, some case studies have revealed a link between dietary nitrate feeding and methemoglobinemia in infants. (Katan, 2009). Nitrate concentrations and bacterial contamination of well water have been associated to neonates' methemoglobinemia (blue baby syndrome) (Sullivan, 2011). Due to higher levels of the methemoglobin-reducing enzyme NADH cytochrome b5 reductase and more acidic stomach conditions, adults tend to be less prone to methemoglobinemia; there are no documented cases of methemoglobinemia in this age range from dietary ingestion of nitrate naturally present in food (Pfiffner, 2017).

Cancer and Dietary Nitrate

According to data found in the 1970s, nitrate can be further changed into nitrosamines after being converted to nitrite (Wolff and Wasserman, 1972). Bacon and sausage that have been cured with nitrate and nitrite may produce nitrosamines when cooked at high temperatures (Li *et al.*, 2012).

Nowadays, a lot of processed meats contain sodium ascorbate and sodium erythorbate, which can assist stop the creation of nitrosamine during cooking and storage (Pfiffner, 2017). Furthermore possible is endogenous nitrosamine production. In the acidic environment of the stomach, nitrite can be protonated to produce nitrous acid (HNO₂), which quickly decomposes to produce powerful nitrosating agents that can react with amines, particularly a-amino acids (such as proline, glycine, and alanine), to produce nitrosamines (García-Santos et al., 2002). While there is no clear relation between cured meats and cancer, many academics have speculated, and some epidemiological studies have revealed an association between nitrite and cancer (Ferrucci et al., 2010). Concerns about the safety of nitrate in food have been raised since these nitrosamine compounds include several carcinogens. When substantial amounts of nitrate and amines are consumed together, numerous nitrosamine production can occur, especially when dietary inhibitors of this formation are low (Pfiffner, 2017). Intake of vitamin C is one such element that may affect how nitrate in the diet affects cancer risk (Dellavalle et al., 2014).

Nitrite acceptable daily intake (ADI)

The nitrite acceptable daily intake (ADI) isn't dependent on nitrosamines or methemoglobinemia. The ADI for nitrite is 0.07 mg/kg of body weight (b.w.) and is based on rodent lung cardiovascular system adverse effects FAO/WHO Expert Committee Food Authorities, 2003). This ADI applies to all sources of dietary exposure, according to the Scientific Committee of Food (SCF) (1997). commodities and animal products contain high quantities of nitrate/nitrite (Iammarino et al., 2013). However, because of the potential for carcinogenic nitroso compounds to occur, the SCF recommends that the amount of nitrite added to food be reduced to the absolute minimum required to produce the preservation effect and assure microbiological safety (Merino et al., 2016).

There were significant differences in meat consumption trends between nations (Linseisen *et al.*, 2002). In Greece, men consumed 79 g of meat on average per day, but in Spain, they consumed 170 g. The range for women was between 47 g in Greece and 106 g in France. Dietary recall

interviews were also employed by the National Examination Health and Nutrition (NHANES) in the US to calculate meat consumption and nutritional intakes between 2001 and 2002. Despite the fact that men's daily meat and poultry intake was on average 198 g and women's daily meat intake was 120 g greater than in Europe (Bowman et al., 2011), the UK Family Food Survey (2013) reported a decrease in the quantity of household purchases of carcass meat (beef, veal, mutton, lamb and pork) from 211 g in 2010 to 182 g in 2013. Data from the National Diet and Nutrition Survey indicate that diets in the UK can be low in nutrients typically found in red meat. The quantities of meat consumed in various forms varied significantly in the European EPIC study. For instance, 83 g/day, or 54% of all meat, was the largest amount of processed meat consumed by men in Germany. In Italy, men consumed the least processed meat—33.5 g/day, or 24% of all meat of any country. Men in Germany consumed the most pork (35 g/day), Italy the most beef (38 g/d), Spain the most lamb (10 g/d), and Spain the most chicken (31 g/d). Over the past 50 years, meat consumption has risen internationally gradually and sharply in some places. These alterations relate to available money (Sans and Combris, 2015).

Alternatives of nitrite and nitrate in meat products

Food preservation has been practised for millennia, and recent years have seen a significant improvement in shelf life and safety thanks to knowledge and technological advancements. Among the natural antimicrobial agents utilised or researched include lactoperoxidase, lactoferrin, ovotransferrin. lysozyme. avidin. bacteriocins, plants, spices, and EOs. Also, there is growing interest in using natural antimicrobials as food preservatives due to the negative short- and/or long-term effects of consuming artificial food preservatives over time (Naidu, 2000). Since synthetic preservatives can cause health issues, replacing them with natural preservatives is much better for humans and the environment (Gokoglu, 2019). In the 1970s, researchers looked at various ways to minimize the use of nitrites and also reduce the risk of nitrosamine poisoning. Some ingredients can, to varying degrees, mimic some of the effects of nitrite, implying that an alternative

curing mixture may be produced, a color, an antioxidant, and an antimicrobial agent would be needed in this mixture (Mulvey et al., 2010). Mediterranean plants may be added to meat and meat products as extracts or EOs in a concentrated form. These technical procedures may be well adopted due to the proclaimed use of natural ingredients, which can reduce oxidation and food deterioration, resulting in clean label products (Granato et al., 2017). To limit human exposure to n-nitrosamines from processed meats like bacon and cooked ham, healthier preservatives should be investigated. Several kinds of research have indicated that using natural alternatives to nitrates and nitrites is both practical and potentially advantageous. Natural preservatives include plant extracts such as herbs and berries (Cantwell and Elliott, 2017). Furthermore, Deda et al., (2007) have demonstrated that adding tomato paste to frankfurter sausages can reduce nitrite levels without impairing processing and quality attributes.

1. Garlic

Garlic (Allium sativum L.), a typical medicinal plant, possesses anticancer, antiviral, antibacterial, antifungal, immune system, and cardiovascular disease-fighting capabilities in addition to its therapeutic and restorative uses (Santhosha et al., 2013). Furthermore, garlic powder and extract are additives with antioxidant activity that are commonly used as a taste enhancer in meat products (Alirezalu et al., 2020). Garlic includes antioxidants tocopherol and organosulfur compounds (Gheisari and Ranjbar, 2012). In less than 10 seconds, a reaction between allin and 2-propenyl-2-propenethiol allinase produces sulphinate or allicin (diallyl thiosuphinate) from garlic (Alirezalu et al., 2020). Their active constituents are organosulfur compounds rich in garlic, such as allylsulfide, diallylsulfide, alliin, and propylsulfide, which have antioxidant properties (Putnik et al., 2019). Benkeblia (2004) reported that sulfur components in garlic had been shown to inhibit Gram-positive and Gram-negative bacteria. After having a nitrate salt and an amine-rich meal, Chung et al., (2002) observed an increase in NMDA, but this production was reduced when garlic extract was added.

2. Thyme

Thyme (Thymus vulgaris) has been used in meat and meat products for centuries (Lawless, 1995). Carvacol, borneol, geraniol, and, most critically, thymol have recently been discovered in their essential oil. Apigenin, naringenin, luteolin, and thymonin are among the flavonoids found in thyme essential oil (Peñalver et al., 2005). These flavonoids increase thyme antioxidant potentials (Kassem et al., 2011). Nguefack et al. (2009) discovered that the volatile oil components of thyme exhibit antibacterial activity against a variety of bacteria and fungi. For example, the activity of thyme has antibacterial demonstrated against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Shigella sonnei (Smith-Palmer et al., 1998). In this regard, Kalemba and Kunicka (2003) discovered that thyme essential oil (500 ppm) has strong antimicrobial properties against foodborne bacteria and fungi such as Aspergillus flavus, Aspergillus parasiticus, Aspergillus ochracus, and Fusarium moniliforme in vitro. Although EOs are natural antimicrobials in vitro, several food components reduce the essential oil's antibacterial activities (Nychas and Tassou 2000).

3. Tomato powder

Tomatoes are solanaceous vegetables that are abundant in lycopene, beta-carotene, ascorbic acid. vitamin E, and phenolic compounds (Eyiler and Oztan, 2011). Lycopene's ability to act as an antioxidant is thought to be responsible for shielding cells from oxidative damage and thereby lowering the risk of chronic illnesses (Kim et al., 2011). Past studies have demonstrated that lycopene and tomato products, such as tomato paste in frankfurters and dried tomato peel in beef hamburgers, are used as antioxidants in meat products (Deda et al., 2007; Selgas et al., 2009; Eyiler and Oztan, 2011). Adding lycopene-rich natural ingredients to minced beef can increase the colour and fibre content in the diet, which has a variety of health benefits, claim Østerlie and Lerfall (2005). Also, tomatoes' acidity prevented the growth of microorganisms. Eyiler and Oztan (2011) found that tomato powder slowed the process and enhanced consumer oxidation acceptability in frankfurters. Furthermore, these scientists discovered that increasing the amount of

tomato powder in the finished product led to increased redness values, consistent with Deda *et al.*, (2007) findings. Tomato powder has also been demonstrated to lower residual nitrite levels in frankfurters while acting as a natural colorant (Eyiler and Oztan, 2011).

4. Smoke liquid

Due to consumer health concerns, smoke liquid has been developed as a safe, natural, and ecologically friendly preservative. After being heated at 400-600°C in a tube or drum and cleaned of hazardous chemicals, the condensation of wood or coconut shell pyrolysis produces smoke liquid (Abustam et al., 2016). There are almost 400 chemical components in this smoke liquid, including phenol (4.13 %), carbonyl (11.3 %), and acids (10.2 %) (Ali and Al Figri, 2020). Smoke liquid contains chemicals that can act as a preservative and emulsifier. Furthermore, various forms of acids serving as gum, which operate as materials thickeners, emulsion stabilizers, and water-soluble gel shapers, were discovered within the smoke (Cahyadi, 2006). Smoke liquid liquid antibacterial properties are linked to the presence of chemicals such as phenols, carbonyls, and organic acids (Vitt et al., 2001). Therefore, several smoke concoctions have been studied to see how effective they kill bacteria (Holley and Patel, 2005). Carbonyl components in smoke contribute to the color and flavor of smoked items, giving them a distinct caramel-like scent. In contrast, acidic components serve as antibacterial agents and contribute to the smoked product's taste (Ariestya, 2016).

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النترات والنتريت والنيتروزأمين في منتجات اللحوم

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المضافات الحافظة للأغذية هي مواد طبيعية أو صناعية تمنع كل من التطور الميكروبي ونشاط الإنزيمات والأكسدة من التسبب في تدهور الغذاء. يستخدم لذلك النتريت (E250 ،E249) والنترات (E251، E251) بشكل متكرر في حفظ اللحوم لما له من له فائدة تكنولوجية كبيرة تساهم في تطوير لون وردي مميز، وهي مسؤول عن إنتاج النكهة المميزة للحوم المعالجة، كما ان لها نشاط مضاد للأكسدة، وتمنع نمو بكتيريا المسببة لفساد الغذاء، وخاصة Clostridium botulinum ، التي تنتج السم البوتشيوليني القاتل، وهي معتمدة من الإتحاد الأوروبي (لائحة الاتحاد الأوروبي رقم EC 2011/2011/1129). ومع ذلك، فإن تكوين الميثيموجلوبين هو أكثر أعراض سمية النتريت وضوحًا وتميزًا في البشر. علاوة على ذلك، يمكن أن تتحد النتريت مع بعض الأمّينات في الأطعمة لتكوين مركبات النيتروزو، وكثير منهّا معروف بأنها مواد مسرطنة. وبسبب الآثار الضارة قصيرة و/أو طويلة المدى لاستهلاك المواد الحافظة الغذائية الكيميائية بمرور الوقت، فان هناك اهتمام متزايد باعتماد مضادات طبيعية للميكروبات كمواد حافظة للأغذية ونظرًا لأن المواد الحافظة الصناعية يمكن أن تسبب مخاطر صحية، فإن استبدالها بمواد حافظة طبيعية أفضل بكثير للبشر والبيئة. ويناقش هذا البحث المرجعي إمكانية استخدام المستخلصات النباتية والدخان السائل والنبات نفسه في الحفاظ على منتجات اللحوم مما ينتج عنه منتجات نظيفة. قد يتم تبني هذه العمليات الفنية بشكل جيد.