Continuing Controversies Regarding Human Health Concerns from Nitrite and Nitrate Consumption in the Diet

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Abstract Nitrite, salt, seasonings and other ingredients are used for the curing to give unique color, flavor and texture to the meat products. Sodium or potassium nitrite is incorporated into the processed meats to provide desirable meaty flavor, prevent warmed-over flavor, develop a bright reddish pink color and inhibit the microbial growth, particularly for out-growth of *Clostridium botulinum* spores. Sodium or potassium nitrate can also be used to cure the processed meats. However, nitrate has to be reduced to nitrite by the microorganisms to be effective for curing and mostly used for the slow-cured products including some fermented sausages and country style hams. The safety of nitrite and nitrate used for meat curing was questioned in the 1970s due to their potential to form carcinogenic nitrosamines in the stomach following the ingestion. Conversely, some potential health benefits were also attributed to both nitrite and nitrate in the recent studies since both compounds contribute to nitric oxide production in human body. Nitric oxide produced directly from nitrite has a significant effect on cardiovascular health by controlling blood flow in the cardiac muscle. Thus, the continuing controversy regarding human health concerns from nitrite and nitrate consumption in the diet are evaluated and discussed in this chapter.

Keywords Nitrate · Nitrite · Nitric oxide · Meat curing · Human health

Introduction

Curing is one of the basic processing procedures used in meat processing. Application of nitrite, salt, seasoning and other ingredients to meat is defined as meat curing in today's technology. Curing is important to give unique color, flavor and texture to meat products and for preventing microbial growth. The salt was originally used at high concentrations for curing and preserving the meat in ancient

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times. The preservative effect of salt is related with the water activity. Salt reduces the water activity of the product and limits the microbial growth. However using only salt produces an unattractive brownish-gray color and often produces too salty meat products. Early meat processors discovered that use of potassium nitrate (saltpeter) found in salt as an impurity fixes and preserves the pink color of cured meat. Although meat curing used to have a sole purpose of preserving meat, today, effects of curing on color development, flavor and textural properties of meat products are equally as important as preservation due to the availability of refrigerators (Aberle et al. 2001; Pearson and Gillett 1996).

Use of nitrite as a sodium or potassium salt in meat curing has several benefits including stabilizing the bright reddish pink color of the lean tissues, contributing desirable flavor of cured meat, preventing development of rancidity and warmed-over flavor, effectively inhibiting the growth of the *Clostridium botulinum* spores. The most important reason for adding nitrite was defined as the effect of the nitrite for preventing the growth of the *Cl. botulinum* in cured meat products. Although sodium or potassium nitrate were originally used for meat curing, microorganisms firstly converts nitrate to nitrite during the processing (Aberle et al. 2001; Pearson and Gillett 1996).

Although use of nitrate has a similar functionality as nitrite, nitrate acts slower and consequently it is used less often. The bacterial reduction is required as an additional step for conversion of nitrate to nitrite. Natural bacterial flora in the meat or added starter cultures that have nitrate reducing capabilities are responsible to reduce nitrate. Nitrate reductases expressed by the bacteria that use nitrate as a substrate for anaerobic respiration include following strains; *Lactobacillus plantarum*, *Lactobacillus sakei*, *Leuconostoc strains*, *Staphylococcus xylosus*, *Staphylococcus carnosum*, *Staphylococcus aureus*, *Bacillus subtilis* etc. (Bonomo et al. 2008; Hammes 2012; Heaselgrave et al. 2010; Majou and Christieans 2018; Talon et al. 1999).

Thus only nitrite is mostly used for obtaining desirable flavor and color and microbial safety in modern meat curing. Limited use of nitrate is still available for some traditional and slow cured products including country cured ham and fermented sausage. Nitrite directly added or converted from nitrate produces nitric oxide which reacts with myoglobin to produce raw cured meat color. This pigment is called nitric oxide myoglobin which has an attractive, bright red color before heat processing. The heat treatment denatures the protein portion of myoglobin and yield a pigment called nitrosylhemochromogen which is responsible for the stable characteristic pink color of cured meats (Aberle et al. 2001; Pearson and Gillett 1996).

Two pathways were described for nitrite reduction to nitric oxide (NO·). The first pathway includes periplasmic (gram negative bacteria) or cytoplasmic bacterial nitrite reductases and second pathway includes nitrite reductase activity of deoxymyoglobin of meat. Myoglobin exists in three different forms in meat including reduced form called deoxymyoglobin, oxygenated ferrous myoglobin called oxymyoglobin or oxidized form called metmyoglobin. The existence of three different forms of myoglobin depends mostly on the partial pressure of oxygen. (Aberle et al.

2001; Gladwin and Kim-Shapiro 2008; Majou and Christieans 2018; Moller and Skibsted 2006; Pearson and Gillett 1996).

In the United States, addition of nitrite is limited to 200 ppm for most cured meat products, 156 ppm for comminuted meats and 120 ppm for bacon depending on the amount of meat and meat by product in the formulation. However, in the European Union the amount of in going nitrite is restricted to 150 ppm for cured products. Addition of reducing agents including the sodium salts of ascorbic acid (sodium ascorbate) or erythorbic acid (sodium erythorbate) to the curing mixture greatly accelerates nitric oxide and further nitric oxide myoglobin formation while the natural reduction is a slow process and is only beneficial for manufacturing of some traditional or slow cured products. Thus use of reductants decreases the time required to develop cured meat color to several hours from several days which is essential step for continuous or accelerated productions (Aberle et al. 2001; Santarelli et al. 2008).

Curing ingredients can easily be incorporated into the comminuted meat products during mixing stage. Dry curing and pickle curing are two fundamental methods used for curing primal or subprimal cuts of meat although there are a number of modified or combined methods available. Dry curing takes longer to cure the meat cuts since curing ingredients including mostly salt, sugar, and nitrite and/or nitrate are added and rubbed on the meat cuts without additional water. Efficacy of this method is determined by the diffusion of salt into the meat. In pickle curing, a brine is prepared by dissolving the same curing ingredients in water. A container is used to submerge the meat cuts into the pickle to allow the curing ingredients penetrate the meat completely. Since pickle curing may require time as much as the dry curing to complete some other methods were developed to increase the rate of curing like artery pumping, single-needle stitch pumping and multiple-needle stitch pumping (Pearson and Gillett 1996).

Concerns Related to Nitrate and Nitrite in the Diet

A continuing debate regarding human health concerns has been going on due to the consumption of nitrite and nitrate in the diet for more than 40 years. Scientific studies have been conducted to determine the safety of nitrite or nitrate in foods and their interactions in the human body after ingestion. High levels of nitrate found in drinking water were associated with infant methemoglobinemia in 1960s (Bryan et al. 2012). In addition, nitrite and nitrogen oxides react with secondary amines and N-alkylamides to produces N-Nitroso compounds which are alkylating agents and they can react with DNA. N-Nitroso compounds include nitrosamines and nitrosamides which have been shown to be carcinogenic to animals in laboratory. N-nitrosamines can be found in tobacco smoke and some foods including certain processed meats (e.g. grilled bacon), smoked fish, cheeses and beers which raised questions about the potential human health concerns (Santarelli et al. 2008).

Nitrite intake from cured meats has been reported to contribute 4.8% of daily intake (Archer 2002). In 1970s cured meats were recognized for their potential for nitrosamine formation under special conditions including presence of secondary amines, available nitrite for reacting, neutral pH values, and high end cooking temperatures of the products (>130 °C) such as fried bacon. In 1978, a final rule was published after discussions of several purposed regulations, stating that use of nitrite was limited to 120 and 550 ppm and sodium ascorbate or sodium erythorbate should be added in bacon. Use of nitrate was banned for bacon production. After implementation of nitrosamine monitoring program all bacon producers were in compliance with specified limits in the regulations (Sindelar and Milkowski 2012).

During the 1970s and 1980s the reactivity of nitrite with nitrosatable amines was investigated to determine their toxicity using animal models. At the same time, manufacturers evaluated the presence of N-nitrosamines in processed foods and beverages and developed new processes and ingredient changes to eliminate or minimize the formation of N-nitrosamines. Thus some modification in brewing methods for alcoholic beverages and use of reductants in cured and processed meats were introduced for the industry. In addition, many countries changed their regulations related to use of nitrite and nitrate in cured meat products to balance toxicological risk with the benefits of using nitrite and nitrate for food preservation and food safety purposes (Bryan et al. 2012).

Daily dietary intake of nitrate is estimated about 43–131 mg by the World Health Organization. Plant derived foods are the primarily exogenous sources (87% of dietary nitrate intake) for human intake of nitrate. Drinking water is also reported as a significant contributor in the total nitrate intake. Beets, spinach, celery, radishes, cabbage, lettuce, and collard greens are among the vegetables contain naturally occurring nitrates in higher concentrations (Archer 2002; National Academy of Sciences 1981; Sindelar and Milkowski 2012; World Health Organization 2007).

Fertilizers cause a greater uptake of nitrogen in vegetables resulting in higher nitrate content. Nitrate content of vegetables is affected by nitrate reductase activity, nitrate uptake, growth conditions and growth rate. In addition some processing methods including heat treatments and storage conditions can reduce the nitrate. For instance, the nitrate content of vegetables decreases with increased storage temperatures due to the reduction of nitrate to nitrite with increased bacterial activity (Chung et al. 2004; Hord et al. 2009; Wolff and Wasserman 1972).

The large portion of total daily ingestion of nitrite (93.0%) was estimated that comes from saliva while only a small portion of the daily nitrite intake comes from foods. The total daily nitrite intake of a person is approximately 1.2–3.0 mg of nitrite per day. Salivary nitrate is reduced to nitrite by the bacteria activity in the oral cavity (Sindelar and Milkowski 2012; World Health Organization 2007).

Most of the gastrointestinal cancers in individuals are known to arise with environmental risk factors rather than hereditary risk factors. Diet is among the strongest contributors related to the environmental factors which increase the risk of gastrointestinal cancer development. Typical Western diets are rich in fat and meat but are poor in fiber content which are related to an increased risk of colorectal cancers. Bile acid secretion increases with a high-fat diet and microflora in colons transforms the bile acid into secondary bile acid which has genotoxic properties to DNA due to nitrogen species and reactive oxygen (Kobayashi 2018; Watson and Collins 2011). Contrary, anaerobic microflora in lower intestine ferments undigested carbohydrate residue consumed in a high fiber diet. These bacteria provide a beneficial environment in the intestine to protect against inflammatory responses and cancer development. However, microbial fermentation of undigested protein residues due to the consumption of protein rich diets is the source of inflammatory and toxic nitrogenous metabolites including amines, phenols, ammonia and indoles. Furthermore, formation of potential carcinogens such as nitrosamine and nitrosamide due to the reaction of nitrosating agents including nitrite and secondary amines and amides are a major risk factor for gastrointestinal cancers (Hamer et al. 2009; Hughes et al. 2000; Lee and Hase 2014). Nitrate is reported as a relatively non-toxic compound below the maximum levels in relations to carcinogenicity. In addition, epidemiological studies indicated that no consistently increased risk of cancer was related with increased nitrate consumption (Alexander et al. 2008; Kobayashi 2018). Colon cancer risk were reported to increasing with chronic exposure to nitrate due to drinking water and food consumption although the risk was associated with low vitamin C consumption while consuming high amount of meat (De Roos et al. 2003; DellaValle et al. 2014). Although fruits and vegetables are the main source of dietary nitrate and nitrite intake, epidemiological and experimental studies are suggesting that the nitrosation inhibitors in the fruits and vegetables have protective effects against cancer development (Bradbury et al. 2014). Furthermore, swallowing saliva with foods has been shown that the major contributor of dietary nitrite intake since 25% of the nitrate ingested in diet is converted to nitrite by oral bacteria after entering the enterosalivary route (Archer 2002; Bryan et al. 2012; Pannala et al. 2003).

Nitric Oxide and Human Health Benefits

Nitrite shown since 1980s is an important molecule for human health. Nitric oxide (NO) is a product of nitrite has so many benefits in human body including controlling blood pressure, wound repair, immune response, neurological functions and blood flow in cardiac muscle as well as preventing some cardiovascular disease like hypertension, atherosclerosis and stroke (Hunault et al. 2009; Sindelar and Milkowski 2012).

An essential role is played by the salivary glands and the oral bacteria to convert nitrate (NO_3^-) and nitrite (NO_2^-) to nitric oxide (NO). NO has important vascular and metabolic functions in the body and is recognized as a messenger molecule. Endogenous production of NO catalyzed by complex NO synthases through the L-arginine pathway. However Nitrate ingested from foods contributes the main extrinsic NO production through NO_3^-–NO_2^-–NO pathway. The salivary glands actively take up to 25% of the nitrate in circulation. Furthermore, nitrate is reduced to nitrite by the oral bacterial species present at the posterior part of the tongue.

Nitrate and nitrite is used by the oral bacteria in their respiration as final electron acceptors (Qu et al. 2016). Since human beings lack of the enzymatic system to convert nitrate to nitrite, bacteria are essential for reducing nitrate to nitrite (Sindelar and Milkowski 2012).

In humans, nitric oxide involves in several physiologic and pathologic processes and identified as an important cellular signaling molecule (Hou et al. 1999; Moncada and Higgs 1993). Nitrate and nitrite ingested by adequate consumption of some fruits and vegetables including beetroot, chervil, lettuce, celery and radish have been shown several potential health benefits for myocardial infarction, acute stress response, hypertension and exercise capacity (Bryan and Ivy 2015; Kobayashi et al. 2015; Machha and Schechter 2012).

Nitrate and nitrite have allowable daily intakes (ADI) of 3.7 and 0.06 mg/kg or 220 and 8 mg/person/day, respectively. The upper small intestine (duodenum and jejunum) absorbs nitrate and nitrite completely and rapidly. Although a small portion of ingested nitrite is converted to NO in the stomach most of the nitrite is absorbed and entered to the circulation. The NO produced in stomach contributes to inactivation of pathogens, mucosal blood flow regulation and formation of mucus. In Addition, enterosalivary circulation of ingested nitrate provides continuous production of NO in the gastric lumen (Bahadoran et al. 2015; Carlstrom et al. 2010; Chow and Hong 2002; Ghasemi and Jeddi 2017; Kevil et al. 2011; Lundberg and Weitzberg 2013; Pannala et al. 2003; Weitzberg and Lundberg 1998).

Nitric oxide (NO) has been reported to have significant roles in mitochondrial respiration, vasodilation, glucose and calcium homeostasis, fatigue development and skeletal muscle contractility**.** The continuous production of NO is important since the half-life of NO is short and ranging from millisecond to seconds. Two pathways have been defined in the human body to generate NO including NO synthase dependent pathway and NO_3^-–NO_2^-–NO pathway which is a NO synthase independent pathway. Three different isoforms of NO synthase have been determined and identified as neuronal, endothelial and inducible NO synthases. L-arginine and oxygen are utilized by these enzymes to generate NO and L-citrulline is also used in a reaction requiring several cofactors comprising nicotinamide adenine dinucleotide phosphate, tetrahydrobiopterin, flavin mononucleotide, flavin adenine dinucleotide, and calcium-calmodulin. Although increased NO production has been reported following the oral supplementation of L-arginine, the bioavailability of NO does not increase in healthy humans who have been consuming pure L-arginine. The end products of NO synthase dependent pathway are nitrite and nitrate since NO is quickly oxidized to form these two anions (Bredt 1999; Dejam et al. 2005; Jones et al. 2018; Moncada and Higgs 1993).

A recently defined NO production pathway involves NO_3^-–NO_2^-–NO pathway with reduction of NO_3^- to NO_2^- and to NO. Green leafy vegetables including beetroot, chervil, lettuce, celery, radish, rocket, and spinach are rich in nitrate containing over 250 mg/100 g of fresh produces that are also exogenous source of inorganic nitrate in the diet. Ingested nitrate reaches the upper gastrointestinal tract where it is absorbed and enters the systemic circulation. After 60 min following the consumption of nitrate, the peak plasma concentrations are achieved. The salivary gland absorbs 25% of the nitrate through sialin which is an active transporter and then nitrate is concentrated up to 20 fold in saliva. Most of the remaining nitrate is either extracted by kidney or excreted in the urine. Facultative anaerobic bacteria in the oral cavity which are positioned on the dorsal side of the tongue plays a major role in reducing approximately 20% of salivary nitrate to nitrite. After the swallowing of the saliva, the nitrite is further reduced to NO in the stomach. The acidic environment in the stomach and the existence of vitamin C and polyphenols greatly enhances the reduction of nitrite to NO. Some part of nitrite also enters the systemic circulation. The peak plasma concentrations occur around 2–3 h after nitrate consumption. This nitrite is quickly circulated in blood and tissue and is reduced to NO in a reaction catalyzed by deoxymyoglobin, deoxyhemoglobin, xanthine oxidase, cytochrome P-450, aldehyde oxidase, the mitochondrial electron transfer complexes, and NO synthase (Benjamin et al. 1994; Duncan et al. 1995; Govoni et al. 2008; Lundberg and Weitzberg 2009; Qin et al. 2012; Wagner et al. 1983; Weitzberg and Lundberg 1998). Hypoxia and acidosis conditions have been shown that considerably enhance the reduction of nitrite to NO indicating a backup system which guarantees continuous NO production even though oxygen dependent NO synthase pathway is not functioning properly. Since the conditions of hypoxia and acidosis are likely to occur during excessive contraction of skeletal muscle, NO production via NO_3^-–NO_2^-–NO pathway may have a specific significance during exercising (Castello et al. 2006; Jones et al. 2018; Richardson et al. 1995).

Dietary nitrate has been reported to decrease blood pressure. When reduction of salivary nitrate to nitrite is interrupted, the decrease in blood pressure could be blocked which is a confirmation of the regulatory function of nitrite converted from consumed nitrate (Aboud et al. 2008; Larsen et al. 2006). Furthermore, sodium nitrate supplementation has been shown that increases the plasma nitrite and reduced oxygen consumption during a submaximal cycling exercise. Similarly consumption of beetroot juice which is a rich source of nitrate for 3 days doubled the plasma nitrite and reduced the oxygen requirements approximately 5% during exercise. Thus, it was indicated that nitrate ingestion with diet allows performing more muscle work per unite time using the same amount of energy with an increased skeletal muscle contraction efficiency. Similar results were also reported for acute nitrate treatment after 60 min of nitrate consumption and 2.5 h of beetroot juice consumption and 15 days of continued nitrate supplementation maintained the increased efficiency during exercise similar to that of acute beetroot juice consumption (Bailey et al. 2009; Jones 2014; Jones et al. 2018; Larsen et al. 2007; Vanhatalo et al. 2010). Although supplementation of nitrate in the diets of people who are moderately active have benefit to improve exercise efficiency, ingestion of nitrate have been reported to not improve exercise efficiency significantly in trained people. Since trained people could have better aerobic fitness and higher resting plasma concentrations of nitrate and nitrite than nonathletic people, improved muscular efficiency seems to be reduced in trained subjects due to already developed oxidative metabolic system and higher bioavailability of NO (Christensen et al. 2013; Jungersten et al. 1997; Longo et al. 2014; Porcelli et al. 2015; Schena et al. 2002).

Conclusion

Curing is defined as an application of nitrite, salt, seasoning and other ingredients to meat products and is an important technological procedure to give unique color, flavor and texture to meat products and for preventing microbial growth. Nitrite addition is the most effective way of preventing the growth of the *Cl. botulinum* in cured meat products. Nitrate is originally used for meat curing however microbial activity is required to convert nitrate to nitrite during processing. Since nitrite can react with secondary amines and N-alkylamides to produces N-Nitroso compounds which have been shown to be carcinogenic to animals in laboratory there has been a continuing debate regarding human health concerns due to the consumption of nitrite and nitrate in the diet for more than 40 years. N-nitrosamines can also be found in tobacco smoke and some foods including smoked fish, cheeses and beers in addition to certain processed meats. During the 1970s and 1980s manufacturers developed new processing and ingredient changes to eliminate or minimize the formation of N-nitrosamines in processed foods and beverages. Thus use of reductants in cured and processed meats and some modification in brewing methods for alcoholic beverages were introduced for the industry. In addition, regulatory changes related to addition of nitrite and nitrate in cured meat products have been placed in many countries to balance toxicological risks and the benefits of using these compound in processed foods. Furthermore, nitric oxide which is a product of nitrite has been shown an important molecule for human health in 1980s. Benefits of nitric oxide in human body includes controlling blood pressure, wound repair, immune response, neurological functions and blood flow in cardiac muscle as well as preventing some cardiovascular disease like hypertension, atherosclerosis and stroke. In addition, nitrate ingestion with diet has been shown to improve muscle work per unit time using the same amount of energy with an increase skeletal muscle contraction efficiency indicating that nitric oxide production via NO_3^-–NO_2^-–NO pathway may have a specific significance during exercising.

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