Nitrite and Nitrate in the Meat Industry

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DESPITE the many published reports on the beneficial properties of nitrite and nitrate in physiology, nitrite and nitrate in cured and processed meats continues to be perceived as harmful. The previous chapter revealed that certain foods, particularly green leafy vegetables are naturally enriched in nitrite and nitrate from growing in soil. However, enriching meats with nitrite or nitrate during curing is perceived as harmful and advocated by some groups to be eliminated completely. The use of pure sodium nitrate in curing is now only a minor practice in the United States. The ingoing levels of sodium nitrite have been tightly controlled by the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture. To satisfy consumer demands for no added nitrite processed meats, efforts have recently been taken to creatively adjust the meat curing process by employing "nitrite free" organic vegetable powders instead of directly adding sodium nitrite salts. Although the end result is production of nitrite from the nitrate contained in the vegetable powders, a more "natural" or "organic" approach seems to appeal to consumers. This is primarily due to the public perception of nitrite and nitrate. Reports about methemoglobinemia in infants (blue baby syndrome) caused by drinks or food prepared with nitrate-rich (and bacterially contaminated) well water and vegetables, intentional and occupational intoxications in adults, increasing nitrate levels in soil and lakes as a result of fertilizer overuse, and the formation of potentially carcinogenic N-nitrosamines all contribute to the negative image that nitrite and nitrate have held in recent years. As a result, major efforts have been made to remove as much nitrite and nitrate as possible from our drinking

water, to advocate replacement of nitrite by other (often less effective) food preservatives, and to establish cultivation conditions that result in crops with reduced levels of nitrate. We feel it prudent at this juncture to discuss the history and use of nitrite and nitrate in meat products and compare to what is contained in vegetables to hopefully dispel the fear of health concerns and the public perception of nitrite in meats.

HISTORY OF NITRITE

Documents dating to around 800 AD suggest that nitrite and nitrate were used by the Chinese medicinally to relieve "acute heart pains, and cold in the hands and feet" [1]. Inorganic nitrite and nitrate have been in use for as long as 5000 years in the preservation of food. The use of salt plus nitrite and/or nitrate in the manufacturing of meat products is commonly expressed as "curing". To cure means to correct, restore or treat so in most cases curing is thought as something positive one could do to meat. Long before the advent of refrigeration, curing and preserving foods from microbial growth was essential and commonplace. In ancient time, curing preserved the meat and fish from spoilage. It was thought in the beginning that the use of salt lowered the water activity and inhibited growth of microorganisms. In the 19th century people realized that some salts were better preservatives than others. Saltpetre (potassium nitrate) was recognized as a contaminant of the salt which enhanced its preserving action and gave meat a red color [2]. Later it was realized that the mechanism underlying food preservation was actually the conversion of nitrate to nitrite by bacteria [3]. This provided the rationale for the use of nitrite rather than nitrate in the meat preservation industry in the early 1900s. Its use is critical for both food safety and palatability. Nitrite in meat greatly delays the out growth of the obligate anaerobe *C. botulinum* and thus development of botulinum toxin, develops cured meat flavor and color, retards development of rancidity during storage, inhibits development of warmed-over flavor and preserves flavors of spice and smoke [3]. Nitrite in food controls and stabilizes the oxidative state of lipids in meat products [4], thus preventing lipid oxidation. Ascorbic acid or dehydroascorbate is also a common meat preservative. It was demonstrated very early on that the chemical basis for the nice red color and appearance of cured meat was the reaction of nitrite with oxymyoglobin to form S-nitrosomyoglobin [5]. It was later realized that free sulfhydryl groups were necessary for this effect [6]. The process was shown to involve intermediate formation of S-nitrosothiols to ultimately produce nitrosylmyoglobin [7]. In the early 20th century meat preservation regulations in the United States for the first time allowed nitrite to be

used as a curative salt. According to the Meat Inspections Regulations, the maximum amount of nitrite that can be used for the curing process is one ounce per 100 pounds of meat (dry cured) or 1/4 ounce per 100 pounds chopped meat and/or meat by-product [8]. Nitrite has since become a common dietary nutrient in those who consume cured meats.

In the 1970s, there became a major public health concern regarding nitrite, when there was indication of endogenous formation of N-nitrosamines from nitrite and nitrate and its relevance to human cancer. The first report in the 1950s on the hepatocarcinogenic effects of N-nitrosodimethylamine (NDMA) [9], and the suggestion that low molecular weight N-nitrosamines (RNNOs) can be formed following nitrosation of various amines [10] ignited an enormous interest in N-nitrosamines and their association with cancer. Proof that nitrosation reactions by nitrite can occur was provided by Ender *et al.* [11] who identified NDMA in nitrite preserved fish, and by Sander and Sief [12] who demonstrated the *in vivo* formation of a nitrosamine in the acidic conditions of the human stomach. Because of the potent carcinogenicity of some low molecular weight N-nitrosamines and the ability of nitrite to form these compounds, considerable effort was made to determine the levels of nitrite and nitrate in the external and internal human environment, and to assess exposure in order to correlate it with human cancer at specific sites [13]. The deductive reasoning from this data concluded that since nitrite can form N-nitrosamines and some N-nitrosamines can cause cancer, then nitrite must cause cancer. Since the early 1980s there have been numerous reports on a possible association of N-nitrosamines and human cancers [13,14] but a causative link between nitrite or nitrate exposure and cancer is still missing [15]. The deductive reasoning does not hold true. In fact, the National Toxicology Program (NTP) which was established by the Secretary of Health and Human Services in 1978 is obligated to provide Congress a biennial report on carcinogens. The NTP consists of the relevant toxicological activities of: National Institute of Environmental Health Sciences (NIH/NIEHS), National Institute for Occupation Safety and Health (CDC/NIOSH) and National Center for Toxicological Research (FDA/NCTR). NTP cancer bioassays are the gold standard which utilize standard protocols, multiple doses of the test compound, extensive tissue pathology and statistical analysis and then draft a report prepared for peer review committee. Their results and recommendations affect regulatory action by the government. The Food and Drug Administration nominated nitrite to be tested by NTP as a carcinogen. Studies were initiated in 1989 looking at long-term carcinogenicity of nitrite. This was a two year study in rats and mice dosed with massive amounts of nitrite in their drinking water [sodium nitrite doses:

750, 1500, or 3000 ppm; (35–150 mg/kg/day in rats and 45–220 mg/kg/day in mice)]. These doses are far greater than one could ever consume through diet. The in-life portion of the study was completed in 1997 with extensive histopathology and statistical evaluation. Draft Technical Report No. 495 was issued in April 2000 for peer review. The results from the report was a unanimous decision of "no evidence" of carcinogenicity in male or female rats and male mice and "equivocal evidence" in female mice. Strongest statistical finding was $p < 0.001$ decrease in "all organs' mononuclear cell leukemia" in male and female rats. To summarize the current state of the art, nitrite, due to its inherent nitrosative chemistry has the ability to nitrosate amines. Some low molecular weight nitrosamines have been shown to be carcinogenic but to date there have been no causal relationship between nitrite and carcinogenicity. It is important to make this distinction as we go forward.
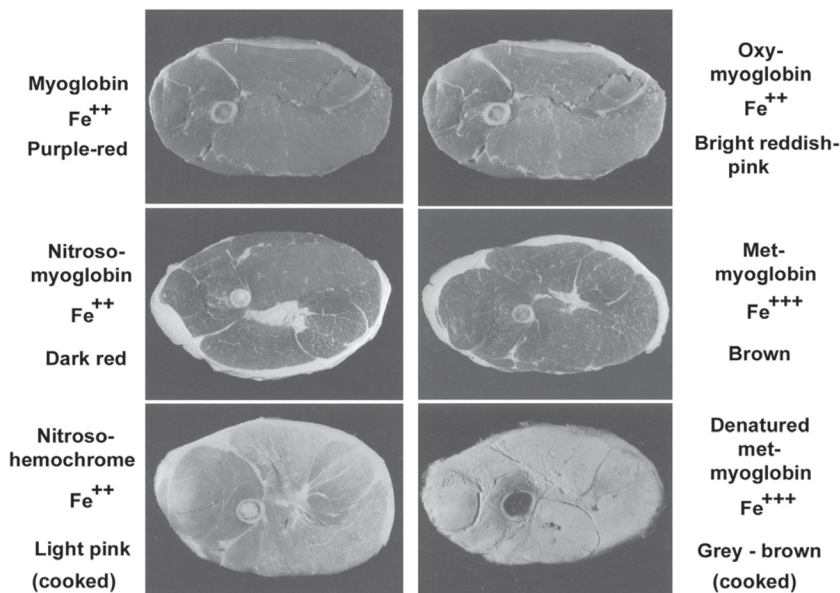
Public awareness was also brought to industrial settings and exposure of workers to nitrite. Not only is nitrite used as a color fixative and preservative in meats and fish but it is also used in manufacturing diazo dyes, in nitroso compounds, in the textile industry, in photography and in the manufacture of rubber chemicals. Nitrite is also a common clinical and laboratory chemical that is used as a vasodilator [16], bronchodilator [17], intestinal relaxant [18] and even as an antidote for cyanide poisoning [19]. Considering its widespread use, there have been many toxicological studies on acute and chronic exposure to nitrite. The fatal dose of nitrite is in the range of 22–23 mg/kg body weight (from USFDA Generally Recognized as Safe Food Ingredient: Nitrates and Nitrites (Including Nitrosamines) 1972 by Battele-Columbus Laboratories and Department of Commerce, Springfield VA). Lower doses of either nitrite or nitrate have caused acute methemoglobinemia, particularly in infants where a high nitrite or nitrate intake has been associated with "blue baby syndrome" caused by methemoglobinemia [20–22]. These negative connotations of nitrite and nitrate have led the government to regulate and restrict the levels in food and drinking water.

In the late 1970s, despite all the fear and paranoia surrounding nitrite exposure, our appreciation and understanding of nitrite took a drastic turn. Studies on nitrogen balance in humans and analyses of fecal and ileostomy samples indicated that nitrite and nitrate are formed *de novo* in the intestine. These early findings by Tannenbaum *et al.* [23] significantly altered our conceptions of human exposure to exogenous nitrite and nitrate and represented the original observations that would eventually lead to the discovery of the L-arginine:NO pathway. Prior to these studies it was thought that steady-state levels of nitrite and nitrate originated solely from the diet and from nitrogen fixing enteric bacteria.

THE CHEMISTRY OF CURING MEAT

Whereas the biomedical community was initially excited about the recently described biochemistry of nitrite, this underlying chemistry has been known and exploited for centuries in the meat industry. Polenske in 1891 [24] from the German Imperial Health Office published experiments where he proved that by adding nitrate only to a pickling solution, nitrite was formed due to the action of microorganisms in the brine. Haldane demonstrated that redox reactions occurred in meats during curing and extracted NO-myoglobin as the substance responsible for the bright pink color of cured meat [25]. It was later revealed that nitrous acid (HNO_2) or a metabolite like NO was the molecule responsible for reacting with myoglobin. This was the first demonstration of the coloring of meat by nitrogen compounds but at the turn of the 20th century the antimicrobial action was still thought to be due to sodium chloride concentrations and not nitrite or nitrate. Color formation and stability are amongst the most critical quality traits of processed meat products. It is
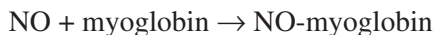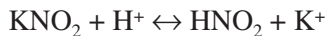
Different States of Myoglobin

Myoglobin Fe^{++} Purple-red

Oxy-myoglobin Fe^{++} Bright reddish-pink

Nitroso-myoglobin Fe^{++} Dark red

Met-myoglobin Fe^{+++} Brown

Nitroso-hemochrome Fe^{++} Light pink (cooked)

Denatured met-myoglobin Fe^{+++} Grey - brown (cooked)

(Modified from Koch Supplies, 1973)

FIGURE 5.1 Scheme of the mechanism of action of nitrate in cured meats.

now known that the characteristic cured color can be derived from the concentration of heme pigments (myoglobin and hemoglobin), their chemical states and additives such as nitrogen oxides and reducing agents. The cured color is a result of the chemical reaction between compounds derived from nitrite/nitrate and the naturally occurring red myoglobin leading to the simultaneous formation of the bright red nitrosylmyoglobin whereby an axial ligand NO is coordinated to the central heme iron [26]. In the last decade ascorbic acid or ascorbate (erythorbate) began to be used in cured meats. Ascorbate reacts with nitrite to form nitrous acid or NO [27]. Ascorbate is also added to inhibit the formation of nitrosamines. By reducing enzymes or chemical reactions with a reducing agent like ascorbic acid the Fe^{3+} is reduced to Fe^{2+}. The NO formed from nitrous acid or N_2O_3 can bind to the myoglobin (Fe^{2+}) and forms a heat stable NO-myoglobin. Oxymyoglobin is not heat stable and dissociates turning the meat grey or brown. On heating NO-myoglobin, the protein moiety is denatured but the pink NO-porphyrin ring system (often called nitroso-hemochrome) still exists and is found in meat products cooked to 120°C. This heat stable pink color will change to brown ("fade") due to oxidation which can be caused by bacterial spoilage or exposure to light and oxygen. The color changes are advantageous as the consumer recognizes spoilage due to changes in color. The figure above illustrates the different states of myoglobin based on nitrite addition.

Potassium Nitrate KNO_3 (saltpeter) → reduction by microorganisms → nitrite (KNO_2)

$$KNO_2 + H^+ \leftrightarrow HNO_2 + K^+$$

$$2HNO_2 \leftrightarrow N_2O_3 + H_2O$$

$$N_2O_3 \leftrightarrow NO + NO_2$$

$$NO + myoglobin \rightarrow NO\text{-}myoglobin$$

Nitrite and nitrate are highly soluble in aqueous solutions especially at pH 5.5 which is the pH of raw meat. Since the pKa of nitrite is 3.37 it can be expected that about 99% of the nitrite exist as an anion at pH 5.5. The small amount of undissociated nitrous acid is in equilibrium with its anhydride N_2O_3 which again is in equilibrium with nitric oxide and nitric dioxide. The NO molecule itself can easily be oxidized to NO_2 in the presence of oxygen. Therefore the oxygen sequestering activity of nitrite renders it as an antioxidant in meat. Due to the lack of oxygen the devel-

opment of rancidity or a warmed over flavor are retarded. The antioxidant effect of nitrite in meats can be attributed to several mechanisms. (1) Fixing a Fe^{2+} heme moiety avoids having a very potent pro-oxidant Fe^{3+} heme present to initiate and promote lipid peroxidation. (2) Nitrite and its equilibrium nitrogen oxides act as free radical scavengers and also prevent lipid peroxidation. (3) The reaction with oxygen scavenges oxygen so it cannot participate in lipid peroxidation. The addition of ascorbate also can result in this antioxidant effect. The oxidation of nitrite to nitrate in meat also explains why nitrate is found in meat products to which only nitrite has been added. In fact nitrite is in some cases found at lower concentrations of nitrate in the finished product (Bryan unpublished observations).

ANTIBACTERIAL PROPERTIES OF NITRITE— AN INGREDIENT CRITICAL TO FOOD SAFETY

The anti-botulinal properties of nitrite have long been recognized. The use of nitrite to preserve meat has been employed either indirectly or directly for thousands of years. In the last 20 years however, the role of nitrite in protecting public health with respect to other pathogens has begun to be better recognized. Nitrite excreted in saliva has significant antimicrobial benefits when it is swallowed and converted to nitrous acid and other nitrogen oxides in the gut. This pathway was described in the preceding chapters. The bacteriocidal effects of gastric fluids are significantly enhanced by the presence of ingested nitrite. This has been demonstrated for known food borne pathogens such as *Escherichia coli* O157:H7 [28,29]. Nitrite and nitric oxide are also effective bacteriocidal agents against other microorganisms associated with diseases such as *Helicobacter pylori* [30], organisms associated with dental caries, [31] and skin pathogens [32,33].

As a food additive, nitrite is a key in controlling potential growth of *Listeria monocytogenes* in processed meats. Models that estimate the effects of ingredients on microbial growth show dramatic reductions when nitrite is included [34–40]. The use of such models have enabled formulations of nitrite cured processed meat products that will not support growth of *Listeria monocytogenes.* To date, this has not been achieved for uncured counterparts where the only ingredient difference is nitrite. The USDA Agricultural Research Service has done extensive research to develop models to predict growth of pathogens under a variety of conditions. In these models incorporation of nitrite at currently used levels significantly inhibits growth of *Listeria, E.coli,* and *Salmonella.*

NITROSAMINE FORMATION

The public health concerns are not related to the molecules nitrite and nitrate but their propensity to form N-nitrosamines in the stomach once swallowed. N-Nitrosamines are a large group of potent carcinogens. Approximately 300 different N-nitrosamines are carcinogenic. At least 30 animal species are responsive to their effects [41]. Human exposure to preformed N-nitrosamines occurs through the diet, in certain occupational settings, and through the use of tobacco products, cosmetics, pharmaceutical products, and agricultural chemicals. Diminishing human exposure to these carcinogens is one approach to prevention of cancer, and this has been accomplished in many instances, although exposure to N-nitrosamines in tobacco products is still unacceptably high. In the 1970s discussion began about the potential formation of nitrosamines in cured meat products, especially fried bacon. Nitrosamines are formed by amines reacting with nitrogen oxide products at very high temperatures or in an acid environment where nitrosative chemistry is predominant. It should be noted that bacon seems to be the only cured meat where this was ever deemed a practical problem due to the temperatures that can be reached during bacon frying. While there have been reports of measurable nitrosamines in other cured meats, they were generally situations not reflecting commercially produced cured meat products made since the late 1970's in the US. Many of the early reports on the formation of nitrosamines by nitrite were in artificial systems co-administering low molecular weight amines with nitrite. Due to the inherent nitrosative chemistry of nitrite in the acid environment of the stomach it is not surprising that such reactions occur. Human exposure to N-nitrosamines occurs by nitrosation of amines in the body, via their acid or bacterial catalyzed reaction with nitrite, or by reaction with products of nitric oxide generated during inflammation or infection. N-Nitrosamines undergo a simple cytochrome P450-mediated metabolic activation step, which is critical for their carcinogenicity. One of the most potent inhibitors of nitrosation reactions is ascorbic acid or vitamin C [42,43]. Substantial reductions in the formation of nitrosamines have been achieved with ascorbic acid and other nitrite scavengers. Ascorbic acid is now routinely added to cured and processed meats in addition to nitrite. There are important considerations to appreciate and understand when discussing the dietary nitrite and nitrate and formation of nitroamines in the stomach. Firstly, amines must be present. In fresh meat there are very minute amounts of amines present. They are creatine, creatinine and the free

amino acids proline and hyroxyproline and some of the decarboxylation products of other amino acids. During aging and fermentation more amines will be formed. Secondly, only secondary amines form stable nitrosamines. Primary amines are immediately degraded to alcohol and nitrogen. Tertiary amines cannot react. Most amines in meats are primary amines derived from α-amino acids. Thirdly, the pH must be low enough to produce NO^+ or metal ions must be engaged to form NO^+ [2]. In products heated above 130°C nitrosamines can be formed. Bacon frying, cured sausage grilling or frying of such cured meat products as pizza toppings are conditions that may lead to formation of nitrosamines. This can be avoided by proper frying, grilling and preparation. It should be noted that even though temperatures of frying/grilling may be high, the evaporative cooling that occurs limits the formation in most products. Bacon is the notable exception because of its high fat content.

NITRITE AND NITRATE IN CONTEXT

The concern about nitrite and nitrate and their potential to react with secondary amines to form carcinogenic nitrosamines resulted in intensive nutritional, biochemical and metabolic research in the 1970's and 1980's. One of the basic approaches in these studies was to measure consumption and excretion of nitrite and nitrate. The results produced an anomaly. Excretion always seemed to exceed consumption [44–46]. This implied synthesis of these compounds in the body and led to a search for the pathways. In 1987, nitric oxide was identified as the transient factor that caused smooth muscle relaxation [47]. It was determined that synthesis of nitric oxide was synthesized from the amino acid arginine as a substrate and that it was degraded into nitrite and nitrite for excretion as a biological control mechanism [48–50]. Typically endogenous nitric oxide is produced at about 1 milligram per kilogram of weight per day in humans. Being a very reactive molecule it is quickly bound to heme and oxidized to nitrite and nitrate. The nitrate is circulated in the blood and can be excreted in the urine, sweat or saliva of the individual. The following chapter will reveal evidence indicating that nitrite itself has a biological function as a signaling molecule independent of nitric oxide [51]. Curiously this function involves nitrosyl and s-nitroso heme species—the very same compounds involved in production of cured meat pigments. In a recent publication a German researcher proposed classifying nitrite as a "prodrug" based on its many newly discovered physiological functions [52]. Clearly, nitrite is a metabolite and is natu-

rally made in significant quantities. Exogenous nitrite ingestion is small by comparison, being at an average residual level of approximately 10 ppm in commercial cured processed meats [53–56]. Although it is not widely realized, potassium nitrate is used in many toothpaste formulations. Toothpaste designed for sensitive teeth is formulated with up to 5% potassium nitrate, (which is 5000 ppm) [57–63]. These products can be purchased at any drugstore.

We know that nitrite in food controls and stabilizes the oxidative state of lipids in meat products [4], thus preventing lipid oxidation. Carr and Frei [64] have previously shown that nitrite inhibits myeloperoxidase mediated LDL modification. Thus the underlying chemistry of nitrite in meats that has been exploited for centuries may then have similar effects in human physiology. In order to gain some insight into the role of nitrite in the context of a high cholesterol diet as would occur when consuming meat, the Bryan lab recently performed a series of experiments in mice. Hypercholesterolemia causes a pro-inflammatory phenotype in the microcirculation. This phenotype appears to result from a decline in NO bioavailability that results from a reduction in NO biosynthesis, inactivation of NO by superoxide ($O_2^{\bullet-}$), or both. Since nitrite has been shown to be potently cytoprotective and restore NO biochemical homeostasis, we investigated if supplemental nitrite could attenuate microvascular inflammation caused by a high cholesterol diet. C57Bl/6J mice were fed either a normal diet or a high cholesterol diet for 3 weeks to induce microvascular inflammation. The mice on the high cholesterol diet received either nitrite free drinking water or supplemental nitrite at 50 mg/L or 150 mg/L ad libitum in their drinking water. The results from this investigation reveal that mice fed a cholesterol-enriched diet exhibited significantly elevated leukocyte adhesion to, and emigration through the venular endothelium as well as impaired endothelium-dependent relaxation in arterioles. Administration of nitrite in the drinking water abrogated leukocyte adhesion and emigration to basal levels, and prevented the arteriolar dysfunction. This was associated with sparing of reduced tetrahydrobiopterin (BH_4) and decreased levels of C-reactive protein. These data reveal novel anti-inflammatory properties of nitrite and implicate the use of nitrite as a new natural therapy for microvascular inflammation and endothelial dysfunction associated with hypercholesterolemia [Stokes et al., *American Journal of Physiology* (in press)]. These data argue for a potent beneficial effect of nitrite in meats or any food products high in cholesterol.

Although the biomedical science community is aware of the emerg-

ing beneficial effects of nitrite, it is still regarded as an undesired food additive in cured and processed meats [65]. However studies reveal that dietary nitrite supplementation can restore NO biochemistry in eNOS$^{-/-}$ mice as well as prevent injury from ischemia-reperfusion insult [66–68], and most recent data reveal that nitrite attenuates inflammation and preserves endothelial function. Emerging evidence from animal models and human clinical studies indicate that, independent of its role as a source of NO in tissues by reduction, nitrite exerts unique intracellular signaling properties that mediate physiologic functions [69], and this is supported by the novel findings that tetrahydrobiopterin (BH$_4$) levels (critical cofactor for NOS enzymes to convert L-arginine to NO) are preserved by nitrite treatment. Because nitrite is a primary biologically active compound resulting from nitrate reduction in tissues, significant physiological benefits may be associated with the provision of nitrate from dietary sources. The United States Environmental Protection Agency put our limit at 1mg/L nitrate in drinking water. Despite the enormous effort over the past few decades to limit or even restrict dietary nitrite consumption due to the potential to form carcinogenic N-nitrosamines, to date there is no conclusive data to indicate that dietary sources of nitrite and nitrate may be unsafe, especially at doses naturally occurring in foods. Since the early 1980s there have been numerous reports on the association of N-nitrosamines and human cancers [14] but a causative link between nitrite exposure and cancer is still missing [70]. In fact, a two year study by the NIH on the carcinogenicity of nitrite conclusively found that there was no evidence of carcinogenic activity by sodium nitrite in male or female rats or mice [71]. Despite this, the negative connotations of nitrite and nitrate remain, and have led the government to regulate and restrict the levels in food and drinking water, particularly in cured and processed meats. However, this view of nitrite may be changing, as evidence is emerging for a protective role for nitrite against different cardiovascular-related disorders. One should not fear the nitrite contained in bacon or hot dogs. In fact, the nitrite in meats may provide vascular protection from the high fat and cholesterol content. It appears that we may have identified a critical component of our diet that many people are missing. In fact the one compound we have been taught to fear and avoid may be saving our life from inflammatory diseases. A 2005 symposium at the US National Institutes of Health highlighted advances in the understanding of nitrite biochemistry, physiology and therapeutics [72]. This is now a well attended conference held every two years highlighting new discoveries in the nitrite arena.

ABOUT THE AUTHORS

Randall D. Huffman, Ph.D.

Randy Huffman is Chief Food Safety Officer of Maple Leaf Foods based at the company's corporate headquarters in Toronto, Ontario, Canada. In this capacity, Dr. Huffman has overall responsibility for leading Maple Leaf's food safety and quality programs across the Company. This involves identifying and assessing global best operating practices, technologies, ingredients and resources that support Maple Leaf's leadership in food safety and quality assurance. Maple Leaf Foods, with annual revenues in excess of $5 billion (CDN), is a leading consumer packaged food company which operates over 90 facilities across Canada and in the United States, United Kingdom, Asia and Mexico.

Dr. Huffman joined Maple Leaf Foods in January, 2009 after serving for 9 years as the Vice President and then President of the American Meat Institute (AMI) Foundation in Washington DC. In this capacity he was responsible for the day-to-day activities of the Foundation, including its research initiatives, industry best practices development and educational programming. The AMI Foundation's food safety research agenda assists AMI members and the industry at large in implementing solutions to food safety and meat quality challenges and serves as the liaison between AMI and various scientific organizations. The AMI Foundation sponsors research and educational programming on the major food safety hazards associated with meat processing. Among various responsibilities, Dr. Huffman has been a part of both the AMI Foundation-led Listeria Intervention and Control Task Force and the Beef Processing Best Practices Task Force that have developed and conducted multiple in-depth training workshops for industry and government.

Prior to joining the AMI Foundation, Dr. Huffman was director of technical services at Koch Industries, Inc., in Wichita, KS, where he managed food safety and product development issues at Koch Beef Company. Earlier in his career, he served as Vice President of Technical Services at Fairbank Farms a fresh meat processing firm based in Ashville, NY. Dr. Huffman received a B.S. in animal science from Auburn University in Auburn, AL; an M.S. and Ph.D. in animal sciences, with specialization in meat science from the University of Florida, Gainesville, FL.

Nathan S. Bryan, Ph.D.

Dr. Bryan was born in Bryan Texas in November 1973. Nathan grew up in small town Texas. He spent his grade school years in Lexington (population 1000) and attended High School in Caldwell (population 3000). He earned his undergraduate Bachelor of Science degree in Biochemistry from the University of Texas at Austin and his doctoral degree from Louisiana State University School of Medicine in Shreveport where he was the recipient of the Dean's Award for Excellence in Research. He pursued his post-doctoral training as a Kirschstein Fellow at Boston University School of Medicine in the Whitaker Cardiovascular Institute. Dr. Bryan joined the Institute of Molecular Medicine, University of Texas Health Science Center in Houston, in June 2006 in the Center for Cell Signaling. He is also a faculty member of the Department of Integrative Biology and Pharmacology and Graduate School of Biomedical Sciences at the UT Houston Medical School. He is an active member of the Nitric Oxide Society, Society for Free Radical Biology and Medicine and the American Heart Association.

Dr. Bryan's research is dedicated to providing a better understanding of the interactions of nitric oxide (NO) and related metabolites with their different biological targets at the molecular and cellular level and the significance of these reactions for physiology and pathophysiology. Attempts are made to identify what particular changes in NO-related signaling pathways and reaction products occur in disease states such as endothelial dysfunction, ischemia/reperfusion, tissue/cardiac protection, diabetes, atherosclerosis and inflammation with the aim of testing their amenability as biomarkers for diagnosis and/or treatment of specific disease.

Current research is directed to understand the interactions of exogenous dietary nitrite/nitrate (NOx) on the endogenous NO/cGMP pathway and how perturbations in each system affect cardiovascular health. This involves mechanisms of nitrite transport and cellular uptake as well as mechanism of nitrite reduction back to NO and via direct NO-independent signaling actions of nitrite. Work in Dr. Bryan's lab involves molecular biology and analytical biochemistry utilizing gas phase chemiluminescence and HPLC in multiple organ systems and blood. Much of the work involves *in vivo* characterization of NO/nitrite

metabolism using a number of transgenic and knockout animal models of disease. Dr. Bryan and colleagues recently discovered that nitrite is a biologically active molecule which was previously thought to be an inert breakdown product of NO production. These findings have unveiled many beneficial effects of nitrite in the treatment and prevention of human disease. These discoveries may provide the basis for new preventive or therapeutic strategies in diseases associated with NO insufficiency and new guidelines for optimal health. Since nitrite and nitrate are common constituents of many fruits and vegetables, changes in dietary habits may have profound effects on many diseases associated with NO insufficiency. NO insufficiency is a hallmark of a number of diseases including cardiovascular disease. The notion that nitrite can recapitulate NO biochemistry and serve as an alternative and compensatory mechanism of NO production is revolutionary. It may also serve as the endocrine mediator of NO signaling. Moreover, the direct signaling actions of nitrite may represent a novel NO independent pathway for nitrite. If our current understanding is true, then an optimal diet may then consist of a sufficient supply of nitrite and nitrate for health and disease prevention. Understanding this pathway will provide the basis for new preventive or therapeutic strategies in diseases associated with NO insufficiency and new guidelines for optimal health. To date Dr. Bryan has published over 20 peer reviewed papers that have been cited over 1000 times. He has been invited to speak at a number of national and international meetings.

Dr. Bryan currently resides in Houston with his wife Kristen and their two sons Grant and Lincoln. The Bryans spend their weekends at their ranch near Caldwell where they raise cattle and horses and enjoy the great outdoors. Nathan enjoys playing golf and calf roping and team roping on his ranch and in competition.

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