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A Review of Nitrate and Nitrite Toxicity in Foods

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A B S T R A C T

Agricultural advancement and population growth have prompted increases in food supplies, and higher crop yields have been made possible through the application of fertilizers. Large quantities of livestock and poultry on farms, along with the accumulation of biomass and agricultural residues, can cause contamination of ground water resources and other water sanitation concerns in both developing and developed countries. Nitrate is mainly used as a fertilizer in agriculture, and because of its high solubility in water, it can create biological problems in the environment. High usage of nitrite in the food industry as a preservative, flavor enhancer, antioxidant, and color stabilizing agent can cause human exposure to this toxic compound. Nitrite is 10 times as toxic as nitrate in humans. Nitrate is converted to nitrite and nitrosamine compounds in the human stomach, which can lead to bladder cancer. In this review, sources of nitrate and nitrite exposure were investigated. Furthermore, the review evaluates standard levels of nitrate and nitrite in different foods, and acceptable daily doses of these compounds in various countries. Finally, we discuss valid methods of nitrate and nitrite identification and removal in foods.

1. Introduction

Research by Estinec and Foster in the mid-19th century showed that nitrate hinders bacterial growth and prevents food spoilage. This discovery opened the path to adding different chemical compounds into foodstuffs. Nitrate has been used as a preservative and flavor enhancer in beef, pork, sausage, and salami. In addition, nitrate has been used to hinder the oxidation of lipids involved in food spoilage, and as a color stabilization factor (1). In food industry, nitrate is added to meat in range of 150–200 μg/ for inhibition of microorganism's growth and color stabilization (2). The role of sodium nitrate in the inhibition of *Clostridium botulinum* spore growth has become well known in the frozen meat industry due to frequent usage (3). Medical science makes special use of nitrate compounds, including sodium nitrate and amyl nitrate as components in intoxication therapy for cyanide poisoning and as an antihypertensive (4). Compounds involved in nitrate metabolism play various physiological and pharmacological roles in the body (5,6). Beneficial effects of nitrate include the prevention of hypertension and cardiovascular disease, and the nitrate present in vegetables and fruit is effective in the reduction of hypertension (7).

However, almost 35% of all cancers show a relationship with diet (8). Nitrate also has applications in the paint, rubber, refining, gold plating, textile dyeing, pesticide, and perfume industries (9).

Nitrate is mainly derived from manure as ammonium, sodium, potassium, and calcium salts, which have annual sales in the millions of kilograms (10). Nitrate is primarily absorbed via drinking water, vegetables, and other foodstuffs and is also produced in the body in small amounts (11). Nitrate is the most important contamination in groundwater, which has significant hazards in humans, poultry, livestock, and in the environment. The concentration of nitrate in shallow groundwater is typically less than 2 mg/l, while in centralized agricultural areas, the concentration of nitrate can exceed 10 mg/l. Wastewater has low concentrations of nitrate, but this amount can reach 30 mg/l in factories' output effluent (12). Nitrate's concentration in drinking water and its effects on humans and animals is mentioned in Table 1 (13).

It seems the conversion of nitrate to nitrite and nitrosamine is the main proof of toxicity in humans (14). Based on many documents, lethal oral doses of nitrite and nitrate for human are established in 80–800 and 33–250 mg/kg body weight, respectively. It is proven that nitrite is formed during reduction of nitrate of bacteria in the saliva and under gastric acid condition in reaction with secondary amine to form the carcinogenic nitrosamine. Consequently, it suggested nitrite is 10 times more toxic than nitrate and its potential as a human carcinogen (Group 2A) by the International Agency for Research on Cancer (IARC) (15). In adults, 5% to 7% of all nitrates that enters into the body is converted to nitrite. The amount of nitrite conversion in young people and persons with gastrointestinal illnesses is greater due to lower stomach pH (16).

Table 1:Toxic effects of nitrate in different concentrations (ppm).

Effect of it in drinking water	Ranges of nitrate (ppm)
Harmless for humans and animals more than 4 ppm indicator of source pollution and environmental problems	0-10 ppm
Harmless for adults and livestock	11-20ppm
Shouldn't be used as drinking water, but the short-term usage of it doesn't have a problems for adults if the food or nutrition source doesn't contain high levels of nitrate	21-40ppm
Is hazardous for adults and young cattle. If the food source contains low levels of nitrate, it is acceptable to use in young cattle.	41-100ppm
Is harmful in every conditions, shouldn't be used	≥100 ppm

The largest factor that led to prioritizing the measurement of small amounts of nitrate and nitrite was that nitrate and nitrite are precursors of nitrosamine compounds (17). Previous studies confirm that nitrates and nitrites are frequent constituents of many foods including vegetables, fresh and cured meats, dairy products, fruits and grains. Existence of nitrites and nitrates in food could be considered as hazardous compounds when oxidation and reduction status in the gastrointestinal tract. These compounds have harmful impact on human health due to its reaction with naturally present secondary amines to form potent carcinogenic N-nitrosoamines. Since N-nitroso compounds are easily formed by the interaction of a secondary amino compound with nitrite and nitrate in foods. Also, there is an explosion of interest in the toxicology of the nitroso compounds in the induction of cancer in many tissues such as lung, kidney, liver, bladder, pancreas, esophagus and tongue, brain, colon and bone, depending on the species(3,7).

In the presence of acid or heat from cooking, nitrosamine is converted into a di-azonium electrophil and similar ions which reacts with biological nucleophile molculs, such as DNA and proteins. The substitution reactions of biomolecules can disrupt normal cell function and lead to cancer or cell death (18). Animal studies in various species have provided reliable evidence for carcinogenesis in multiple tissues, such as the colon, bronchus, stomach, and pancreas, due to nitrosamine compounds (19,20). The teratogenic effect of nitrate has caused an acceptable daily intake (ADI) to be determined for nitrate and nitrite (21). The most important human and livestock disease caused by the presence of nitrite in food or water is methemoglobinemia, or blue baby syndrome. In this disease, nitrate in the digestion system leads to the production of nitrite. The high aqueous nitrite concentration allows it to pass easily from the digestive tract into the blood. In the blood, nitrite oxidizes the iron in hemoglobin, converting it to iron(ш), which creates methemoglobin, a brown pigment unable to transport oxygen. This process disrupts oxygen flow to the body's tissues, and in the absence of oxygen, the cells of the body become disabled, turning the skin, lips, and nails gray. Other symptoms of this illness include diarrhea, vomiting, lethargy, and malaise, and the last stage of the disease leads to suffocation and death (22,23).

The methemoglobin reductase enzyme is produced in adults, which converts this compound to oxyhemoglobin. Due to a lack of this enzyme, however, several groups of people are still vulnerable to methemoglobinemia, including children, pregnant women, gastritis patients, and people with a congenital deficiency for this enzyme. As a result, nitrate and nitrite in food has been associated with increased risk of stomach cancer and intestinal disease in adults, and methemoglobinemia in infants. The methemoglobin level in the body is usually between 1% and 3%. When the concentration of methemoglobin reaches more than 1.5 g/dl, or more than 10% of an adult's hemoglobin concentration, it becomes important clinically. The lethal dose of nitrate absorbed through food has been determined to be 330 mg/kg of body weight (24).

2. Nitrate and nitrite standards

The widespread use of nitrite in foods, especially meats, has resulted in an average concentration of nitrate and nitrite residues in processed meat that is reported to be 180 mg/kg, compared to 65 mg/kg in other meats (25). Due to the aforementioned risks associated with these ions, many countries and international organizations have set limitations on nitrate and nitrite usage. The World Health Organization has stated that the maximum permissible concentration of nitrate and nitrite in drinking water should not exceed 3 mg/l (25).

The United States Environmental

Protection Agency (EPA) has set a limit of 45 mg/l in drinking water, while the European Union's limit is 50 mg/l. In Iran, 45 mg/l of nitrate in water has been set as the maximum allowable concentration (27).

According to Iran's standards, the permissible concentrations of nitrite are 150 µg/ml for meat products and 500 µg/ml for raw meat (26). At the present time, in America and Britain, the maximum amount of absorbed nitrate deemed permissible to be absorbed on a weekly basis has been determined as 400 to 450 mg/l, which includes 210 mg/l via vegetables, 110 mg/l by meat consumption and 85 mg/l by drinking water. Microbial contamination may have a significant role in making individuals sensitive to nitrate. All drinking water resources should be tested for microbial contamination, particularly if nitrate levels exceed 10 mg/l. The simultaneous presence of nitrate and microbial contamination can indicate inappropriate water well conditions and a probability of pollution from surface drainage, sewer systems, livestock, and other sources (27). The ADI for nitrate has been determined to be 0 to 7.3 mg/kg body weight, and the ADI for nitrite is 0 to 0.07 mg/kg. In the Netherlands, nitrate concentrations in lettuce have been reported to range from 40 to 5500 mg/kg, while the amount of nitrate in spinach was estimated at 30 to 6000 mg/kg. Average nitrate concentrations in other vegetables have also been reported, including 1900 mg/kg in chicory and 1800 mg/kg in beets.

In Britain, the concentration of nitrate in the lettuce varied between 50 and 5300 mg/kg, while in spinach, the concentration of nitrate was 25 to 4600 mg/kg. Nitrate concentrations have been reported to be less than 3 mg/kg in scallions and 180 mg/kg in radishes (25).

Table 2:The amount of the nitrate and nitrite in Middle east's food (25).

Food stuffs	**The total amount of absorption**		**Absorbed**		**Consumption**		**Concentration**	
	Nitrite	**Nitrate**	**Nitrite**	**Nitrate**	**Nitrite**	**Nitrate**	**Nitrite**	**Nitrate**
Potato	0	3	0.0041	1.1	5.9	5.9	0.7	180
Vegetables	7	2	0.14	0.93	230	0.5	0.6	1900
Cereals	54	11	1.1	3.44	430	430	2.6	10
Fish	0	0	0.0016	0.016	3.3	3.3	0.5	5
Fat	1	1	0.021	0.21	341	41	2.6	5
Bird eggs	2	0	0.038	0.072	14	14	0.5	5
Fruits	5	1	0.1	0.048	200	19	0.5	25
Milk	5	1	0.1	0.58	200	120	0.5	5
Cream	0	0	0	0.17	0	8.5	0.5	20
Nuts	0	0	0.0005	0.01	1	1	0.5	10

Table3:Estimated intake of nitrate and nitrite in different parts of the world(25).

Region	**ADI source**						**ADI**		**Absorption amount**	
	Vegetables	**Fruit**	**Water**		**Cereals**					
	NO_3	**NO_3**	**NO_2**	**NO_3**	**NO_2**	**NO_3**	**NO_2**	**NO_3**	**NO_2**	**NO_3**
Middle east	-	5	30	20	55	10	50	20	0.5	40
Far east	45	10	30	30	60	15	50	10	2	28
Africa	30	10	40	40	50	15	40	10	1.6	20
Europe	90	5	20	5	35	-	40	70	1.7	155
Latin America	60	10	35	15	40	5	10	25	1.6	55

3. Measurement methods of nitrate and nitrite

Because of the importance of measuring and controlling amounts of nitrate and nitrite in the environment, it has been essential to develop new, sensitive, and simple ways to measure these ions. Correspondingly, much research has been done in this field. There are several main methods for measuring nitrate and nitrite.

Colorimetric methods and spectrophotometry are the oldest and simplest identification methods. Spectrophotometry methods are simple, effective, and widely available, but have restrictions in their determinations of nitrate and nitrite in non-uniform materials and in high levels of color. Also, in the ultraviolet region, interference from organic materials can cause errors in measurement (28). Chromatographic methods are frequently and widely used for the identification and measurement of chemical compounds in complex mixtures. In this method, mixture components can be passed through a stationary phase by a moving phase of flowing gas or liquid, and separation results based on the differences in the migration rates of fixed and mobile phase components. Gas chromatography, high performance liquid chromatography, and ion chromatography are the most common chromatography methods for determining the amounts of these anions. However, complex derivation methods, expensive columns, and the need for experienced operators have limited the efficacy of these methods despite their high precision (29). Electrochemical methods, luminescence spectroscopy, and fluorescence methods are rarely mentioned in the literature as diagnostic methods (30).

The method used for measuring nitrate and nitrite in Iran's national standards organization is the spectrophotometric method (31).

4. Removal methods for nitrate and nitrite

Appropriate methods of nitrate removal from water include reverse osmosis, distillation, ion exchange systems, and electrodialysis, all of which can require large amounts of capital to use. However, boiling water, carbon absorption filters, mechanical filters, softening water, and adding chlorine do not remove nitrate or nitrite from water. During the distillation process, which involves heating water to its boiling point, collecting the vapors, and condensing them, nitrate is removed. Solely boiling water does not decrease the nitrate concentrations, but the collection and condensation processes are capable of doing so. In reverse osmosis process, water is passed through a semi-permeable membrane using pressure, and as water crosses through the filter, nitrate contamination is eliminated. According to the previous study, nearly 85% to 95% of nitrate can be removed by reverse osmosis. Ion exchange processes exchange calcium and magnesium ions with sodium ions. In the nitrate removal process, the anions with resins that are exchanged prefer sulfate to nitrate. The amount of sulfate in the water is one of the important factors in the effectiveness of the ion exchange system when removing nitrate. All of the above methods are relatively expensive due to setup and operation costs (13).

5. Conclusion

The high usage of nitrate and nitrate in the food industry has necessitated methods for identifying and determining their amounts in foodstuffs. The consequences of a high daily intake of these compounds, and their roles as precursors of nitrosamine compounds, have put them in the spotlight. The most important disease in human and livestock associated with nitrite's effect in water or food is methemoglobinemia. Children, pregnant woman, people with gastritis, and people that congenitally lack methemoglobin reductase are at risk of this disease when exposed to nitrate and nitrite. The consequences of nitrate and nitrite usage in food include increased risks of gastroenteritis in adults and methemoglobinemia in children. Today, it is generally recognized that drinking water, vegetables, and other foodstuffs are main sourcesofnitrateand nitrite, and the toxicity ofthese compounds necessitates continuous monitoring systems for all food sources.

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