Sudan University of Science and Technology

College of graduate studies

Spectrophotometric Determination of Nitrite content in Cured Meats

تقدير محتوى النتريت بالميطيافية الضوئية في اللحوم المعالجة

A Thesis Submitted in Partial Fulfillment for the Requirements of a Master Degree in Chemistry

By

Manal Mohammed Abd Alshafea Ahmed

(B.Sc (Honours), Chemistery)

Supervisor: Dr. Omer Adam Mohammed Gibla

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إستهلال

بسم الله الرحمن الرحيم

(وَكُلُوا مِمَّا رَزَقَكُمُ اللَّهُ حَلَالًا طَيِّبًا وَاتَّقُوا اللَّهَ الَّذِي أَنْتُمْ بِهِ مُؤْمِنُونَ)

(المائدة اية 88)

Dedication

To my parents,

brothers and sisters

Acknowledgment

My endless praise is due to Allah Almighty for giving me health strength and success to perform this work.

My great thanks go to my supervisor Dr. Omer Adam Mohammed Gibla for his continuous encouragement and help during his supervision of the work. My thanks would also extend to my colleagues of National public health laboratory for their technical support, cooperation and help. My thanks would include all those who gave me help and encouragement during my work in this study.

Abstract

The aim of this study was to determine of nitrite ions (NO^-_2) in some locally produced, processed meats samples. Twenty three samples were obtained from the local markets in Khartoum state. The cured meat samples include Sausages, Martidala, Beef berger, Basterma, Hot dogs and Frankfurters. Nitrite ions were extracted from the samples by hot water. Nitrite content for each sample extract was measured by Uv-Vis spectrophotometry. Three samples showed nil nitrite content. Twenty samples showed nitrite content within the permissible range (130mg/Kg) according to national regulation for food additives (Sudan, 2006). The highest mean values were founded in Hot dog samples (6.88mg/kg) and the lowest mean values were founded in Sausages samples (0.66mg/kg). The concentration was expressed as sodium nitrite ($NaNO_2$).

المستخلص

تم في بعض عينات اللحوم المعالجة التي تصنع محليا.(NO^{-}_{2}) هدفت هذه الدراسة لتقدير ايونات النتريت الحصول على ثلاث وعشرين عينة من اسواق ولاية الخرطوم وقد شملت العينات (السجوك, المارتديلا, البيرقر البقري, الباسطرما, الهوت دوق والفرانكفورتر). تم استخلاص ايونات النتريت من العينات باستخدام الماء الساخن. تم قياس محتوى النتريت المستخلص من كل عينة بجهاز ميطيافية الضوء المرئي وفوق البنفسجي. نتائج التحليل اظهرت خلو ثلاث عينات من النتريت. عشرون عينة اظهرت تراكيز في اعلى تركيز لوحظ (2006) 130) وفقا للمواصفة السودانية لمضافات الاغذيةmg/kgالمدى المسموح به(. (0.66mg/kg) وادني تركيز في عينات السجوك بمتوسط (6.88 mg/kg)في عينات الهوت دوق بمتوسط قدرت التراكيز في صورة نتريت صوديوم ($NaNO_2$.)

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CHAPTER ONE

Introduction

1. Introduction

1.1 Meat preservation

1.1.1Historical background

Meat and food preservation was an essential development, so, that early hunter gatherers could maximize the harvest and extend the food supply by delaying spoilage. Freezing, salting, and drying of foods was used by early humans, and the method of preservation was depends on the surrounding environment (Wentworth 1956). Sun drying was initially, developed as an effective preservation practice in areas of low humidity. Later meat was dried and smoked over or alongside fires. Freezing, as method of preservation, was limited to geographical climate and season. On the Arabian Peninsula and in coastal regions; early civilizations discovered salting meats was an effective preservation technique. Natural nitrate deposits are found in parts of the world and it can be formed on walls of building covering nitrogen rich soils .In the 1840‘s, nitrosation of amines was first identified and research continued to discover other nitrosated/nitrosylated compounds .Cured meats, as known today, are believed to have originated from preserving meat with nitrate contaminated salts. It is unknown when nitrates were first intentionally used but the earliest records of nitrate‘s reddening effect date to the 10th century, late in the Roman Empire. During the late1890‘s, researchers noted that nitrite, not nitrate, was responsible for cured meat characteristics. Identification of cured pigment was reported in 1901. Much research has been conducted to investigate the characteristic color, flavor/ aroma, antioxidant activity, and antimicrobial activity associated with cured meats (Sullivan, 2011).

1.2Cured meats

For centuries, meat has been preserved with salt. At certain levels, salt prevents growth of some types of bacteria that are responsible for meat spoilage. Salt prevents bacterial growth either because of its direct inhibitory effect or because of the drying effect it has on meat. Most bacteria require substantial amounts of moisture to live and grow (Epley, Addis, and Joseph J, 1992).

Cured meat has specific properties including a pink color and characteristic flavor and texture (Borchert and Cassens ,1998).

Curing is the treatment applied over food or other products, to prevent decay by the addition of salt or smoke. The curing process refers to food preservation by drying or removal of water. It is difficult to track the exact origin of the curing practice in human civilization, as it has been present in history for thousands of years (Roberts, 1996). The main purpose of curing was to preserve the food in times where refrigeration was not available, and was especially important for meat products (Redondo-Solano, 2011).

1.3 Curing agents

Sodium nitrite and potassium nitrate have a long history of use as curing ingredients, and by the close of the 19th century the scientific basis of the process was becoming understood. It was realized, for example, that nitrate must be converted to nitrite in order for the curing process to proceed. These additives have two purposes. One is “cosmetic”, they give the meat a pinkish-reddish color and prevent it from turning brown. The other is that they prevent the growth of clostridium botulinum, the microorganism that produces the deadly botulism toxin (Borchert and Cassens, 1998).Sodium nitrite ($NaNO_2$) is commonly added to meat products which are kept for an extended period in a cold, but not frozen state. This compound is produced from nitrogen oxides or nitrous fumes that are obtained

after the catalytic oxidation of ammonia with air in acontrolled environment. The nitrogen oxides are then absorbed in of sodium carbonate or sodium hydroxide solutions to obtain pure, white to yellowish color (Redondo-Solano, 2011).The chemical reaction is shown below:

$NO + NO_2 + 2NaOH$ (or Na_2CO_3) $\rightarrow 2NaNO_2 + H_2O$ (or CO_2)

Nitrite, in comparison to nitrate, is a much more reactive compound. Like nitrate, the pKa of nitrous acid, HNO_2, is relatively low, 3.3. Most of this compound would be found as the nitrite anion, NO_2^-, in biological or meat curing conditions but some of nitrous acid would be found. The nitrite ion must be reduced to act as anitrosating/nitrosylating agent. Acidification of nitrite provides one of the best methods to form nitric oxide. In the presence of mineral acids and other reducing compounds, nitrite can be non-enzymatically reduced to nitric oxide. However, the anhydrous form of nitrous acid, dinitrogen trioxide, N_2O_3, is thought to be the one of the main nitrosating compounds. Reduction of nitrite in meat curing systems is essential to provide nitrosation/nitrosylation reactions and is impacted by many factors including pH, temperature, endogenous compounds, and other added ingredients. In vivo,the nitrite produced by bacteria in the oral cavity is readily reduced to nitric oxide in the acid conditions of the stomach and provides a supplement nitric oxide source(Lundberg et al 2008).

Early researchers noted the color change of hemoglobin with the addition of nitrite. This reaction is now known to form nitosylmetmyoglobin which rendershemoglobin unable to transport oxygen. Cyanosis caused by ingesting sodium nitrite or nitrite containing food has been reported throughout medical literature (Sullivan, 2011).

Later, several studies demonstrated the presence of high amounts of nitrosamines in cured products, when subjected to high temperatures. There are more than 300 different types of nitrosamines and about 97% of them have been shown to be

teratogenic in laboratory animals. (Redondo-Solano, 2011). The chemical process that leads to the formation of nitrosamines in meat systems is depicted below.

$NaNO_2 + H^+ \rightarrow HNO_2 + Na^+$

$HNO_2 + H^+ \rightarrow \square NO^+ + H_2O$

$2HNO_2 \rightarrow N_2O_3 + H_2O$

$N_2O_3 \rightarrow NO + NO_2$

$NO + M^+ \rightarrow NO^+ + M$

Primary amine $RNH_2 + NO^+ \rightarrow RNH\text{-}N = O + H^+ \rightarrow ROH + N_2$

Secondary amine $R_2NH + NO^+ \rightarrow R_2N\text{-}N = O + H^+$

Tertiary amine $R_3N + NO^+ \rightarrow$ no nitrosamine formation

This chemical process involves the same reactions leading to the formation of nitric oxide and nitrous acid. Therefore, the same conditions may lead to the reduction of nitrite which favor nitrosamine formation. The reactions involve primary, secondary or tertiary amines, with secondary amines forming the more stable nitrosamines. Amines are present in very low concentrations in fresh meat products in the form of creatine, creatinine or free amino acids like proline or hydroxyproline (Honikel, 2008).

Nitrosamine formation happens, in vivo, if nitrites and other precursors are acquired from the diet as already demonstrated in human subjects. The latter means that the concern regarding nitrosamines is related to both the nitrite fraction that already reacted with meat components and the residual nitrite. Nitrite itself can be toxic if consumed in very high quantities; it can interact chemically with hemoglobin and interrupt the normal oxygen transportation in humans (Redondo-Solano, 2011).

Today, the use of sodium nitrite is acknowledged by regulatory authorities around the world as an important contributor to food safety. In addition, a rapidly

expanding body of scientific research is demonstrating that the advantages of nitrite extend well beyond food safety to include a diverse and critical array of potent benefits for human health.

Nitrite concentrations decline in meat mixtures during processing. Many factors influence the rate of nitrite reduction in meat, Gary Anthony Sullivan (2011) and Greenwood (1940) proposed six factors that influence nitrite loss:

1. Time and temperature employed during processing
2. Amount of protein, fat, and carbohydrate
3. Concentration of salt
4. Concentration of nitrate and nitrite
5. Number of microorganisms
6. Acidity

1.3.1Cured Meat Color

The pink color of cured products is a product of the reaction between nitrite and compounds present in the meat (Montville and Mathews 2008).The fixation of a desirable red color, shaded pink, is the most obvious effect from nitrite addition and is often considered an extremely important attribute for consumer acceptance. Interestingly, very little nitrite is needed to induce a cured color. It has been reported that as little as 2 to 14 ppm, depending on species, is necessary to induce a cured color (Sindelar and Milkowski2011).

The formation of color is the most understood reaction in meat curing. Research of reaction on nitrite and heme pigments has been conducted since the 1860‘s and nitric oxide hemochromogen was identified as the cured pigment at the turn of the 20th century. Over the next several decades, the mechanisms ofthe redox reaction of myoglobin and nitrite or nitric oxide were described. A stepwise mechanism has been proposed for cured color development. Myoglobin reacts with nitrite to form

met myoglobin and nitric oxide which then react to form nitrosyl metmyoglobin. Scientists debate whether cured meat pigment is found as mononitrosyl hemochromogen or dinitrosyl hemochromogen. Increased salt content has been shown to increase the rate of nitrosyl myoglobin formation. The addition of S-nitrosocysteineto meat can produce typical cure color and other cured meat characteristics suggesting its role as an intermediary nitrosylating/nitrosatingcompound. Satisfactory and stable cured color development can be achieved with the addition of 40 ppm of sodium nitrite. Exposure to oxygen and light results in cured color fading although the presence of sufficient residual nitrite and reducing compounds slows this process (Sullivan, 2011).

1.3.2Flavor and Aroma

The reactions responsible for cured meat flavor and aroma are not fully under stood but it is thought to be primarily related to the limited volatile compounds than their equivalent uncured cooked meats alcohols, phenols, esters, furans pyrazines, aldehydes and other nitrogen containing compounds, and increased carboxylic acids, sulfur, and nitrite/nitrate containing compounds were found in cured versus uncured meat . Less than half of the total volatile compounds were found in cured meat products and much of the differences are thought to be due to the limited formation of lipid oxidation by products. Alcohols and phenols all undergo nitrosation reactions and also could impact volatile compounds. Increases in sulfur compounds are likely due to S-nitrosothiol formation and reduction to disulfide bonds during meat curing. The antioxidant role of nitrite, explains the reduction of oxidation products such as hexanal in cured meats. Further work needs to be conducted to more fully understand the reactions and volatiles responsible for cured meat flavor and aroma (Sullivan, 2011).

1.3.3Antioxidant Activity

The increased oxidative stability of cured meats has been well established. Many reactions that take place in cured meats can extend product shelf life. Lipid oxidation can be initiated by many methods and once started; it exponentially increases by free radical reactions. Oxygen and other reactive oxygen species rapidly react with, and are sequestered by, nitric oxide. Nitric oxide, as a free radical, can also terminate lipid auto oxidation. Nitric oxide binds free iron and stabilizes heme iron which can reduce lipid oxidation by limiting prooxidant activity of the iron. Unsaturated fatty acids are targets of lipid oxidation and the nitrosation of double bonds also could decrease lipid oxidation. The addition of 50 ppm of sodium nitrite has been shown to reduce lipid oxidation products by nearly 65% (Sullivan, 2011).

1.3.4 Antimicrobial Activity

Higher concentration of nitrite is required for antimicrobial activity than to provide other cured meat characteristics. Nitrite has the unique ability to inhibit outgrowth of *Clostridium botulinum* spores and historically has been the primary pathogen of investigation in studying nitrite‘santimicrobial impact. More recently, *Listeria monocytogenes* has been of concern in ready to-eat meats due to ability to grow in high salt and at refrigerating environments. In addition, many nitric oxide-donating compounds have been studied and have shown antimicrobial activity similar to that found in cured meats. It has been proposed that nitrite targets bacteria at multiple sites by inhibiting metabolic enzymes, breaking the proton gradient, and limiting oxygen uptake .Synthetic iron-sulfur complexes react with nitric oxide and form complexes similar to Roussin‘s black and red salts . Nitric oxide binding to iron regulates and limits iron availability which is necessary for enzyme functionality and bacterial metabolism and growth. Due to high reactivity

of iron and nitrite, iron sulfur complexes and heme iron centers of enzymes are often the targets of nitrite. Cysteine is found in many enzymatic processes and signaling pathways and is thought to be another target of nitrite for inhibitory effects. Many ingredients used in processed meats have synergistic antimicrobial effects with nitrite. Increased ingoing nitrite has shown to increase the antimicrobial activity of organic acids. Similarly, lower levels of sodium chloride are needed for inhibition of Clostridium botulinum toxin production when nitrite is added. Anaerobic and more acidic environments increase the antimicrobial effectiveness of nitrite. Gary Anthony Sullivan (2011) and Tompkin (2005) were identified many factors that impact the antimicrobial activity of nitrite and effect product safety and shelf life:

-pH of the product during abuse

- Injection level
- Residual nitrite at point of abuse and the rate of depletion during abuse
- Amount of viable botulinal spores and vegetative cells at the time of abuse
- Temperature of abuse
- Concentration of ascorbate or isoascorbate
- Concentration of available iron in the product
- Type of meat and other formulation ingredients
- The thermal process applied to the product
- The growth of competitive flora

1.4 Dietary Sources of Nitrates and Nitrites

Much confusion has existed about where our oral intake of nitrate and nitrite actually comes from. There are three main sources of dietary nitrate and nitrite:

1. Most of nitrates and nitrites we consume are from the nitrate present in leafy vegetables. Because nearly all vegetables contain nitrates ranging from 0.001 to 1.0%, which accumulates in plants from the uptake of soil nitrogen in the soil during the growth period. When nitrate containing vegetables are consumed, some nitrate will be converted to nitrite by the bacteria found in human mouth. Part of the swallowed nitrate may be stored in human body until it will be needed.
2. Most waters contain small amounts of nitrate and nitrite, depending on the amount drunk. This can be a major source rather than from processed meats.
3. Cured meat products also serve as a small source of nitrate and nitrite (5%). Nearly (90%) of nitrate and nitrite added to cured meats is broken down and converted to other safe compounds. Levels of nitrate and nitrite present in cured meats at the time of storage and purchase are usually between 0.00002 and 0.004% (Sindelar, 2012).

1.5 Antimicrobial additives

Antimicrobial preservatives (Table 1.1) prevent degradation by bacteria. This is one of the most traditional types of preserving. Ancient methods, such as, pickling and adding honey to prevent microorganism growth by modifying the pH level. The detailed mechanism of these chemicals rangefrom inhibiting growth of bacteria to the inhibition of specific enzymes (Lück and Lipinski, 2002).

Table (1-1): Antimicrobial additives

E number	Preservative	Comment
E201 – E203	benzoic acid, sodium benzoate	used in acidic foods such as jams, salad dressing, juices, pickles, carbonated drinks, soy sauce
E214 – E219	hydroxybenzoate and derivatives	stable at a broad pH range
E270	lactic acid	-
E249 – E250	nitrite	used in meats to prevent botulism toxin
E251 – E252	nitrate	used in meats
E280 – E283	propionic acid and sodium propionate	baked goods
E220 – E227	sulfur dioxide and sulfites	common for fruits
E200 – E203	sorbic acid and sodium sorbate	common for cheese, wine, baked goods

1.5.1 Antimicrobial aspects of nitrites in cured products:

Nitrite is an antimicrobial compound with a bacteriostatic effect against a wide range of microorganisms. Conditions present in the meat system that influence antimicrobial properties of nitrites include pH, redox potential,chemical composition of the sample and the temperature of storage (Roberts, 1996). botulinum and other putrefactive microorganisms (Benjamin and Collins, 2003). Since then, nitrites have been used as an approach to prevent the occurrence of botulism from meat products. However, nitrites are also active against other spore forming pathogens. *perfringens* and *Bacillus cereus*, and also against many spoilage microorganisms. Nitrite can control spore formers by inhibiting the outgrowth of germinated spores at the concentrations usually present in meat products (100-200 ppm). Spore viability is affected at much higher nitrite concentrations than the normal amounts used in meat products.

The antimicrobial properties of nitrite have been demonstrated and studied for aerobic and anaerobic microorganisms. In the case of aerobic bacteria the main site of antimicrobial activity of nitrite is the cell membrane and all the metabolic processes associated with this structure. However, the precise site of antimicrobial action is dependent on the bacterial species. For *Escherichia coli* and *Pseudomonas aeruginosa* the main site of inhibition is the enzymealdolase. In the case of *S. aureus*, strong evidence suggests that inhibition occurs in the sulphydryl groups of the coenzyme A or at lipoic acid cofactors involved in pyruvate metabolism. Antimicrobial nitrite effect has been also demonstrated against *Achromobacter spp., Enterobacter spp., Flavobacterium spp., Micrococcus spp., Pseudomonas spp. and Listeria monocytogenes*.

The inhibition of pyruvate metabolism observed in aerobic microorganisms is related to the inhibitory effect observed for anaerobic species. perfringens with both nitrite and nitric oxide.. The role of NO consists in the reaction with non-heme iron centers of many enzymes such as ferredoxin and hydrogenase. The affected enzymes are part of some metabolic steps during glycolysis via the Embden-Meyerhof pathway and the pyruvate metabolism, an important source of energy for the cell. Other indications of the antimicrobial mechanism of nitrite have been elucidated from human physiology studies. Dietary nitrite plays a major role in defense mechanisms in the stomach against enteric pathogens, through a process dependent on oxidative reactions under acidic conditions that leads to the formation of toxic peroxynitrite.

Inhibition of microbial growth by nitrites depends on the organoleptic conditions of the meat system evaluated as well as the type of microorganism involved. A concentration above 150 ppm is considered appropriate to prevent the occurrence of C. botulinum spores germination and outgrowth and food spoilage by putrefactive microorganisms (Roberts, 1996). The relation between nitrite concentrations and microbial inhibition under specific conditions has been studied in terms of residual nitrite. Christiansen et al. (1973) concluded that input nitrite was more important than residual nitrite to predict the safety of canned cured ham using different nitrite levels from 0 to 500 ppm. Christiansen reassessed the influence of residual nitrite levels on antibotulinal action. In cured products stored for long periods of time lower residual nitrite levels were related with a higher risk of botulism in cured products that are temperature abused Christiansen et al., 1978 reported that residual nitrite levels are essential for proper antibotulinal effect and high levels of reducing agents (isoascorbate) may decrease the inhibitory power attributed to these compounds; the same author suggested that residual nitrite may

interact with iron in metabolic enzymes that are essential for microbial survival. Reduction in residual nitrite can also result in a higher dissociation of the toxic nitric oxide from iron in the meat system. Depletion in residual nitrite levels over the storage of sausages increases microbial spoilage by aerobic microorganisms. Higher levels of residual nitrites are correlated with lower concentrations of L. *monocytogenes* in cured products that are vacuum packed and stored at 5 C. *botulinum* inhibition was observed when residual nitrite concentrations remained high at the presence of sorbic acid.

1.6 Monitoring of nitrite in cured products

Factors that influence nitrite chemical behavior complicate analytical quantification in cured meat products. Efforts are made in order to achieve a good correlation between residual nitrite levels, the history of the product and the implications derived from the variations. After the confirmation of the carcinogenic properties of nitrosamines and their presence in cured products in the 1960s, meat processors started changes in the curing process, including a significant reduction in the input nitrite levels and the incorporation of ascorbates (Scientific Panel on Food Additives, 2003). The final result has been a significant reduction in nitrite content in cured products. Monitoring studies have been performed in Europe, the United States and Canada. Studies made in Germany showed that most of the tested cured products (about 50%) contained no detectable nitrite levels; in the cases where nitrite was detected the average content was around 20 mg/kg which is well below the regulatory standard of 175 mg/kg . In Canada, from 1972 to 1996, a survey reported residual nitrite content ranging between 28 and 43.6 mg/kg in different products including bacon, ham, sausages, bologna, corned beef and others. Bologna, frankfurters and various uncooked cured products contained the highest levels of residual nitrite among the products

tested. Low levels of residual nitrite were found in more than 70% of cured samples in Belgium in a study made from 2002 to 2003. Higher variability was found in the United Kingdom in 1997; the residual nitrite was between 0.2 and 123 mg/kg for bacon and 0.2 to 170 mg/kg for other products. Low levels were found by Cassens in the United States (1997).

1.7 **Regulations controlling the use of curing agents**

Many countries have developed specific, regulations to control the amount of nitrate and nitrite present in meat products. Most of the regulations specify the amount of nitrite that must be added to the product as part of the manufacturing process (Roberts, 1996). To control the amount of nitrite input in most of the products and for the possibility for in vivo nitrosamine formation, some regulations established limits for both the ingoing and residual nitrites. The European Parliament and Council published a revised directive (2006) regarding the use of nitrates and nitrites in cured products including residual nitrite regulations (Redondo-Solano, M. S, 2011).

Curing agents were established in the USA in 1926 (see USDA, 1925; USDA, 1926), and the same rules are in effect at present, with slight modification. The critical feature of these rules is that a maximum use level of sodium nitrite is defined; but the meat processor may use less. Basically, no more than one-quarter ounce (7.1 g) may be used per 100 pounds (45.4 kg) of meat (resulting in 156 mg/kg or 156 ppm). While nitrate is still permitted, it is, in fact, not used by the industry. The regulations were changed for bacon so that ingoing nitrite is targeted at 120 ppm, and the maximum use of ascorbates (550 ppm) is mandated. The current routine use of ascorbates (ascorbic acid, sodium ascorbate, erythorbic acid and sodium erythorbate) by the meat processing industry is important not only

because it accelerates and improves the curing process but also the use of ascorbates inhibits nitrosation reactions which might result in formation of carcinogenic nitrosamines.

One possible chemical hazard involved in producing processed meats would be an error in the use of sodium nitrite. If too much is added there is a risk of illness, even death, of the consumer. USDA recognized this concern when the regulations permitting the direct use of sodium nitrite were established. Levels of use and safeguards in handling it were established (Borchert and Cassens, 1998) .

The level of nitrite analytically detectable in cured meat is greatly reduced from the amount added because the nitrite reacts with components of the meat during processing and storage. Modern-day cured meats at retail have a residual nitrite content of about 10 ppm .

The National Institute of Occupational Safety and Health (NIOSH, 1997) reported that there are data indicating nitrite is a primary irritant, tumorigen, mutagen and that it causes reproductive effects. The levels cited for toxic and lethal doses are in the range of those previously referenced.

The National Academy of Sciences (1981) concluded there is no definitive evidence to suggest that either nitrate or nitrite is carcinogenic. In animals, nitrate has not been shown to be directly carcinogenic or mutagenic.

The limited data on nitrite indicate that it may not act directly as a carcinogen, but that it is mutagenic in microbial systems.

Also of interest, at this point, is an understanding of what is considered an acceptable intake of nitrate and nitrite by humans. The FAO/WHO Joint Expert Committee on Food Additives (JECFA) set an acceptable daily intake (ADI) of 0-5

mg/kg body weight for sodium nitrate and an ADI of 0-0.2 mg/kg body weight for sodium nitrite. In other words, they could be manufactured for different applications or for specific formulations, so as to make them optimally useful for a variety of products and applications. Germany and other European Community countries recognized the concern associated with keeping and handling a chemical such as sodium nitrite. They have mandated that only diluted forms of sodium nitrite such as those in pre-blends or curing salt mixtures be allowed in meat processing establishments. (Borchert and Cassens July, 1998)

1.8 Nitrite Debate

During the 1950s and 1960s, observational studies indicated the potential for nitrites to form carcinogenic *N-nitrosamines* in food. N-nitrosamine exposure can occur also through other foods, occupational settings, cosmetics, tobacco products and agricultural chemicals.

In the case of cured meats, *N-nitrosamine* formation may occur when secondary amines react with nitrous acid produced from nitrite at very high temperatures for example, during the frying of bacon at 170C. As potential public health concerns are related to the formation of *N-nitrosamines* rather than to the nitrite itself, regulations were introduced in the 1970s that both limited the addition of nitrite to cured meat products and required the inclusion of *N-nitrosamine*formation inhibitors in bacon. When added to cured meats, ascorbic acid (vitamin C), erythorbic acid (a mirror image of vitamin C) and alpha-tocopherol (vitamin E) inhibit the potential formation of nitrosamine.

In 1980, an Interagency Working Group convened by the U.S. Food and Drug Agency concluded there was no evidence that increased incidences of cancer

tumors of the lymphatic system were induced by the ingestion of sodium nitrite. In 1981 and 1982, this finding was supported in two reports issued by a special committee of the U.S. National Academy of Sciences.

During the 1990s, epidemiological investigations into nitrite, nitrate and nitrosamine exposure and health outcomes were published. Epidemiological studies use public health databases in an endeavour to establish statistical relationships between many factors and human health. One series of studies reported that the consumption of cured meats could be related to cancer and leukemia. These conclusions were reported by the media without complete explanations of the study limitations, uncertainty, or conflicting evidence.

The U.S. National Toxicology Program, a multiagency program supported by many agencies in the U.S. Department of Health and Human Nutrition Services, and the world's leading authority on the toxicological safety of chemicals, conducted a multi-year study to evaluate the safety of nitrite. Constituting original experimentation that assessed the potential of a direct cause and effect relationship, this study became known as the "gold standard" of research on this subject. The study, peer reviewed by a panel of independent experts in May, 2000, determined that nitrite is safe at the levels used in the meat industry and at levels consumed through the diet.

In 2006, a review of various epidemiological investigations that endeavoured to assess the potential carcinogenicity of nitrate and nitrite was conducted by a working group convened by the International Agency for Research on Cancer (IARC). The working group concluded that: "ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans." In other words, under certain specified conditions, ingested amines and

amides can be nitrosated to form carcinogenic nitrosamines. This report did not acknowledge that, as most ingested nitrite is formed in saliva, the swallowing of saliva in combination with virtually any food could be considered to result in the potential formation of nitrosated compounds.

In 2007, a report from the World Cancer Research Fund (WCRF) included a recommendation to limit red meat and eliminate processed meat consumption. These recommendations were considered by others to have been based on weak epidemiological associations and were challenged by researchers both associated with and independent of the meat and poultry industry (Sindelar and Milkowski, 2012).

The aim of this study is.

- To extract nitrite ions using hot water.
- To quantitatively asses nitrites in meat products using spectrophotometric analysis.

CHAPTER TWO

Materials and Methods

2. Materials and Methods

2.1 Collection of samples

Twenty three locally processed meat samples were collected from Khartoum stats markets. The samples include six types of cured meats, namely (Sausages, Martidala, Hambergar, Basterma, Hot dogs and Frankfurters) were collected from locally markets in Khartoum stats.

2.2Chemicals

- Sodium nitrite

Assay not less than 99.9%, Analar analytical reagent, BDH Chemicals Ltd Poole ,England

- Zinc acetate

Assay not less than 99.5%, Analar analytical reagent, BDH Chemicals Ltd Poole,England

- Borax

Assay 99.0 -103%, Laboratoryrasayan , Mumbai

-Activated charcoal

- Sulphanilamide

Assay 89%, BDH laboratory reagents, England

- N-1 -Naphthyleethylene-diaminedihydrochloride

BDH limited poole, England

- Potassium ferrocyanide

Assay not less than 99.0% ,Analar analytical reagent, BDHChemicals Ltd Poole , England

2.3Instruments

-Sensitive balance (Model: ADAM AAA 250LE _ENGLAN D)

-Ultra violet - visible spectrophotometer (Analytikjena Model 07745 -UV/visible spectroscopy single beam, Germany)

2.4 Methods of analysis

2.4.1 Preparation of standard nitrite solution

0.150g of sodium nitrite ware weighed accurately by using a sensitive balance, dissolved in water and quantitatively, transferred to 1000ml volumetric flask. The volume was then completed to the mark and well shaken (1ml =100 µgNO^-_2). Series of lower concentration were then prepared from the stock solution.

2.4.2 Extraction of nitrite from the samples

About ten g of each sample were accurately weighed in 250ml conical flask, 100 ml hot water (80C),5 ml borax solution and 0.5 g activated charcoal were added .The mixture was transferred to a water bath at (70 C) for 15 min .After not less than an 2 hour, 1 ml potassium Ferrocyanide (10.6%), 1 ml zinc acetate (21.6%) and 5 ml borax(5%) were added .Then the contents of conical flask were transferred to a 250 ml volumetric flask . After allowed to standing for 30 min, make up to the mark with distilled water, mixed and filtered through a filter paper.

2.5.2 Determination of nitrite concentration

Twenty ml of each, sample, filtrated were pipetted into a 50 ml volumetric flask, diluted to approximately 40 ml, 5 ml of sulphanilamide (0.5%) were added and allowed to stand for 3 mins. 2 ml coupling reagent was then added, After 20 min the optical density was measured at 540 nm in a 1 cm cell. The optical density of blank solution was also measured.

CHAPTER THREE

Results and discussion

3. Results and discussion

3.1Results

Table (3-1): Concentration of sodium nitrite in Sausages samples (mg/kg)

Sample No	Concentration of sodium nitrite Mg/Kg
1 2 3 4 5 6	0.46 1.78 0.54 1.22 Nil Nil
mean	0.66

Table (3-2): Concentration of sodium nitrite in Martidala samples (mg/kg)

Sample No	Concentration of sodium nitrite Mg/Kg
7 8 9 10 12	2.83 3.00 1.32 0.69 Nil
mean	1.49

Table (3-3): Concentration of sodium nitrite content in Beef berger samples (mg/kg)

Sample No	Concentration of sodium nitrite Mg/Kg
13 14 15 16	1.17 1.58 1.94 0.97
mean	1.42

Table (3-4): Concentration of sodium nitrite in Basterma samples (mg/kg)

Sample No	Concentration of sodium nitrite Mg/Kg
17 18 19	1.34 0.91 1.62
mean	1.29

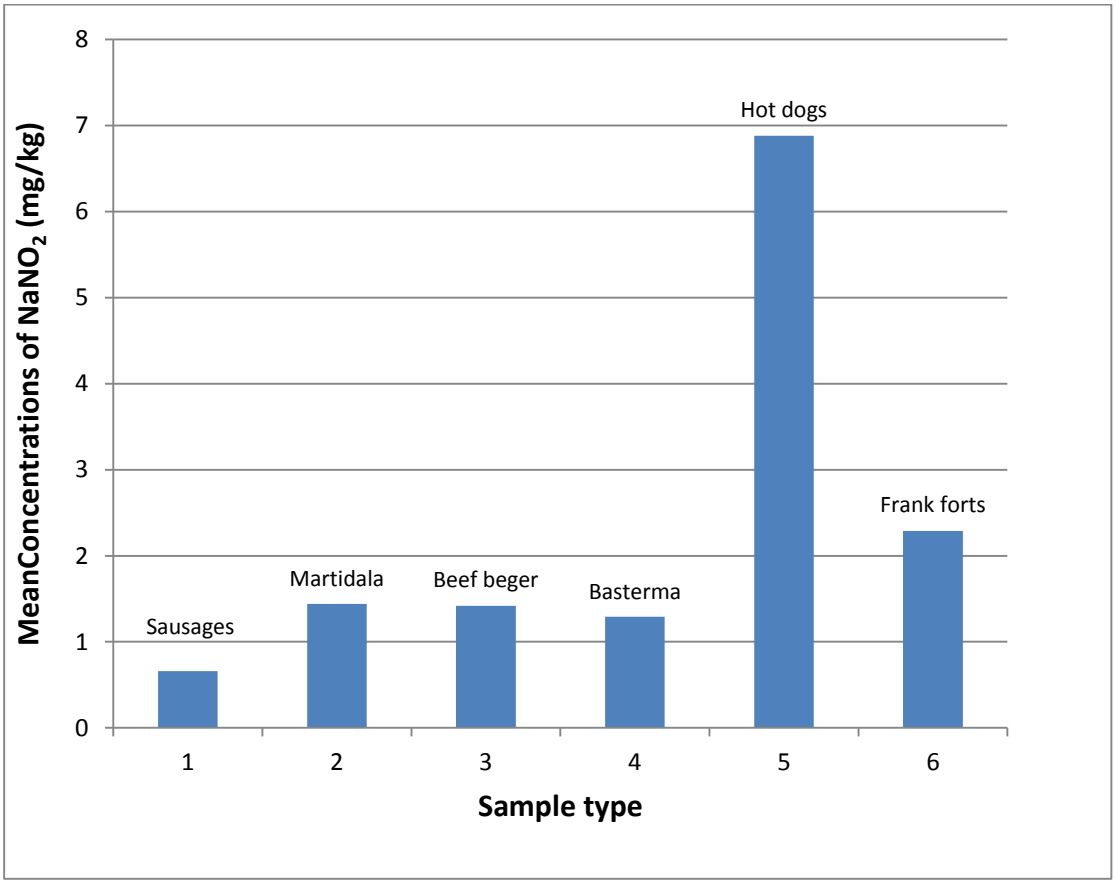
Table (3-5): Concentration of sodium nitrite in Hot dogs samples (mg/kg)

Sample No	Concentration of sodium nitrite Mg/Kg
20 21	12.74 1.01
mean	6.88

Table (3-6): Concentration of sodium nitrite in Frankfurters samples (mg/kg)

Sample No	Concentration of nitrite Mg/Kg
22 23	1.18 3.4
mean	2.29

Fig (3-1): Mean concentrations of samples (mg/kg)



3-2 Discussion

Sausages samples (Table 3-1) showed the highest sodium nitrite concentration in sample (No. 2) and lowest no sodium nitrite concentration in samples (No. 5) and (No. 6). The mean value of sodium nitrite concentration was 0.66mg/kg. All concentrations are within the range according to national regulation for food additives (2006).

Table (3-2) shows the highest concentration was observed in sample (No. 8) and the lowest one in sample (No. 11), sample (No. 12) did not show nitrite content. The mean value was within the permissible range (1.49 mg/kg).

Table (3-3) shows a highest sodium nitrite concentration in sample (No. 15) and lowest concentration in sample (No. 16). The concentrations of sodium nitrite in all type samples (13-16) were within the accepted range with mean value of 1.42 mg/kg.

Nitrite concentration in Table (3-4) had the higher value in sample (No. 19) and lower value in sample (No. 18). All samples here showed concentrations in the permissible range, with a mean value of (1.29 mg/kg).

The samples in Table (3-5) were only Tow. One sample (No. 20) showed sodium nitrite concentration higher than the second sample (No. 21). The two sample from different sources. This type of processed meat may need further investigation dealing with greater number of samples.

Table (3-6) showed higher sodium nitrite in sample (No. 23). The lowest concentration was in sample (No. 22). The mean value is (2.29 mg/kg).

The mean concentration of the six types of samples (Fig 3-1) indicated considerable nitrite content.

Conclusions

. Most of the analyzed samples showed a presence of sodium nitrite.

. From consumer's safety point of view, the presence of nitrite in food is a hazardous factor, regardless of its permissible range.

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