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Determination Of Nitrate in Different Types of Soft Cheese in Libyan Market

تقدير النترات في أنواع مختلفة من الجبن الأبيض في الأسواق الليبية

A Thesis Submitted in Partial Fulfillment of The Requirements for Master's Degree of Science in Chemistry

Submitted by:

Najla Ebrahem Alsiweee

Under the supervision of:

prof. Abdulhafid Elbelazi

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بسم الله الرحمن الرحيم

"وما أوتيتم من العلم الا قليلا"

سورة الاسراء الآية 111

Dedication

To Allah for her kind help

To the soul of my beloved father

To my mother

To my supervisor

To anyone who gives a hand to accomplish this work

Acknowledgment

My first thanks go’s to Allah

A particular thanks also go’s to each one in my family for their helpful help to me.

I am grateful to my supervisor prof. Abdulhafid Elbelazi for his patience and his helpful encouragement and constructive suggestions during the disquisition of my MSc study, also I would like to thank the Alsadeem company specially Dr. Tariq, and the last thanks go’s to my husband.

Abstract

A total of 39 samples of Libyan imported and local white cheese were examined. Samples were taken at randomly from Tripoli markets.

The first nine analyzed samples were spread cheeses, cream cheeses, mozzarella cheeses, feeta cheeses and semi soft cheeses, the Results obtained for analysis of nitrate at each sample were presented separately, Nitrate level was between 3.494 mg/kg to 174.20 mg/kg in all types of cheese samples with an average 6.07 mg/kg. In spread cheese nitrate level was between6.988 mg/kg to 37.934 mg/kg, with an average 15.112 mg/kg, in cream cheese nitrate level was between 20.46 mg/kg to 174.20 mg/kg ,with an average 48.32, in mozzarella cheese nitrate level was between 3.49mg/kg to 40.18 mg/kg, with an average 23.17mg/kg, while nitrate level in feeta cheese was between 151.24 mg/kg to 171.20 mg/kg, with an average 158.22mg/kg and in semi soft cheese nitrate level was between 20.59mg/kg to 50.16 mg/kg,with an average 33.55 mg/kg.

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Abbreviation

E251	Nitrate sodium
E252	Nitrate potassium
IUPAC	International union of pure and applied chemistry
BMD	Bone mineral density
AOAC	Association of official analytical chemists
CPE	Carbon paste electrode
MNP	Magnetic nanoparticles
EDTA	Ethylenediaminetetraacetic acid
NED	N-(1-naphthyl) ethyl enediamine
NAS	National academy of sciences
WHO	World health organization
FAO	Food and agriculture organization of the united nations
IC	Ion chromatography
EU	The European union
MDL	Method detection limit
DC	Direct current
GSO	Geosynchronous satellite orbit
G.C.C	Gulf cooperation council
EOS	Egyptian organization for standardization and quality
SD	Standard deviation

Introduction

1.1. introduction

There are increasing concentration of nitrate level in food products such as meat, cheese, fish, and different kinds of foods.

It has been found that cheese is among the most consumed foods in daily meals, as it adds a wonderful taste to various dishes. However, most types of cheese contain microbial contaminants, which are known as microbial contaminants. These contaminants are produced if a preservative is not added to the cheese. Therefore, preservatives such as sodium or potassium nitrates, which are given food additive numbers E251 and E252, respectively, are added to reduce or prevent their presence. Cheese also helps prevent the appearance of swelling, which improves its appearance, shape, and prolongs its shelf life.

The amount of nitrate in forges and water, particularly if it has been contaminated by nitrate, is mostly determined by the quality of feed provided to cattle and can be discovered in row milk and loading.

In general, nitrate poses little hazard to both people and animals. Its reduction to nitrite is primarily responsible for its toxicity; in the digestive system and silva, bacterial reduction acts as a biotransformation mechanism to convert nitrate to nitrite [1].

Moreover, the nitrite presented in stomach due to nitrate -nitrite contained food intake (such as cheese). Nitrosation can happen during food product storage and ripening; nitrosamines have been detected in cheese even without the presence of nitrate. May react with both secondary and tertiary amines and amides to generate N-nitroso compounds, which have the potential to be carcinogenic.

Furthermore, excessive intake a quantity of nitrates and nitrites are found in diet may cause toxic effects associated with the formation of methemoglobinemia, the named blue baby syndrome which produced by oxidation of hemoglobin by nitrite syndrome.

There are several methods to convert nitrates to nitrites, and the specific method used depends on the surrounding conditions and the purpose of the conversion. here are some common methods:

- Chemical reduction: a reducing agent such as zinc or iron is added in an acidic medium to convert nitrates to nitrites. this process involves transferring electrons to the nitrates to reduce them to nitrites
- nitrates are heated to high temperatures to facilitate the decomposition of nitrates and formation of nitrites. this method is commonly used in industrial processes to produce nitrites.
- enzymatic decomposition: some microorganisms use enzymes to reduce nitrates to nitrites. this method is important in environmental systems such as soil and water

So, measurement of nitrate detected in food is crucial to have more knowledge about nitrate food intake.

1.2. NITRATE

The polyatomic ion nitrate has the chemical formula NO_3^-; nitrates are salts that include this ion. Nitrates are frequently found in explosives and fertilizers [2]. Nearly every inorganic nitrate is soluble in water.

systematic IUPAC name nitrate

chemical formula NO_3^-

^{1}molar mass 62.004 g.mol^{-}

conjugate acid Nitric acid

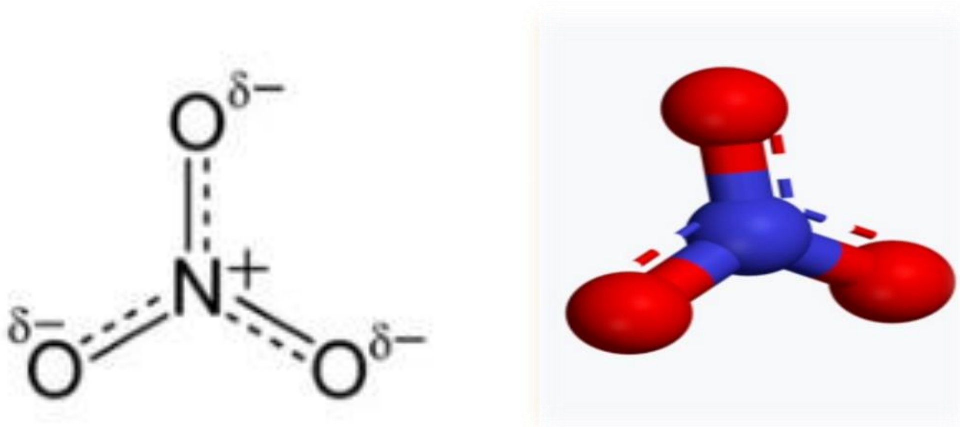
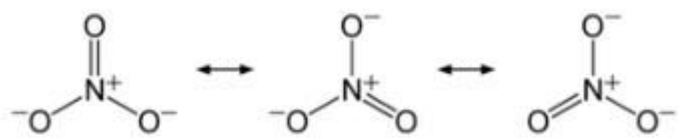


Figure (1)-structure of nitrate

The conjugation base of nitric acid, or ion, is made up of three similarly bound oxygen atoms arranged in a trigonal planar pattern around a single central nitrogen atom. The formal charge of the nitrate ion is -1. This charge is the consequence of a combination formal charge, where the formal charge of the polyatomic nitrate ion is made up of the +1 charge for nitrogen and the -2\3 charge for each of the three oxygen atoms. This composition is frequently cited as an illustration of resonance. Nitrate ions are similar to isoelectronic carbonate ions in that they can be represented by:

resonance structures: 

1.2.1Nitrates salts

Table. 1 some nitrates salts (sodium and potassium nitrate)

Preparties	**Sodium nitrate**	**Potassium nitrate**
Chemical formula	$NaNO_3$	KNO_3
Molar mass	85.00	101.11
Color	Yellow or little white crystals, powder.	White or colorless Crystalline Powder.
Taste	Little salty	Salty and acidly
Fusibility point c°	271	334
Boiling point c°	320	400
Density	2.168	2.109
Dissolution	Liquid in air, soluble in water, little soluble in alcohol.	Apiary In glycerol and water, little apiary in alcohol.

1.3. source of nitrate

Leafy greens like spinach and arugula are major sources of inorganic nitrate in human diets. NO_3^-, or inorganic nitrate, is the active ingredient that can be found in beet root juice and other plants [3].

The salts of nitrites and nitrates are commonly used for curing meat and perishable produce [3], many processed meats are high nitrates· While these nitrates are useful for preserving and improving the color of food; they are not good for human health. Numerous studies suggest adding vitamin C to cured meats high in nitrates to stop the production of dangerous nitrite compounds [1].

others, they are added to food to preserve it and also help hinder the growth of harmful microorganisms, in particular clostridium botulinum, the bacterium responsible for life -threating botulism, while nitrates are used to prevent certain cheeses from bloating during fermentation. nitrate is found the highest concentration in water, due to its use in intensive farming methods livestock production [1].

xanthine oxidase is an enzyme that is naturally produced by cows and is found in milk. It is sometimes utilized for preservation. A portion of this enzyme ends up in their cheese and milk. This enzyme is vital to a cow's health and plays a significant role in their regular metabolic activities.

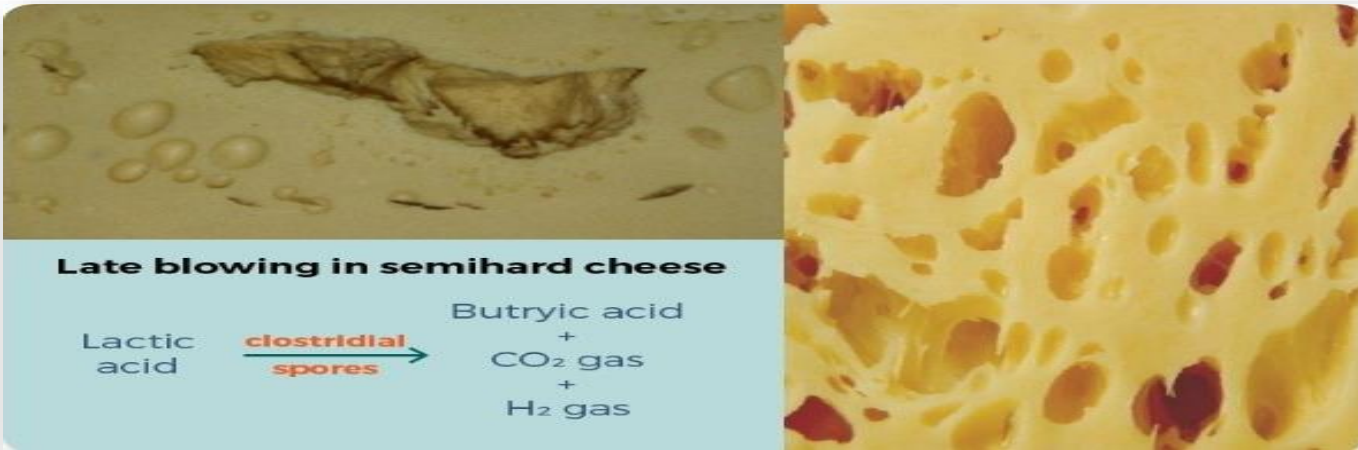


Figure (2)-late blowing is a common defect in many semihard and alpine- style cheeses

this enzyme can aid in the inhibition of clostridia-specific bacteria when coupled with sodium nitrate. Why is this relevant? Clostridium tyrobutyricum is one of the clostridium microorganisms that can produce the "late blowing" fault in cheese. This villain can release butyric acid and gas, which can lead to ugly cracks, openings, and other defects (not to mention a terrible smell). late-breaking semi-hard cheese

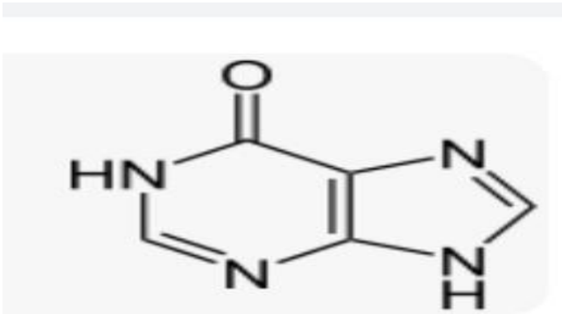
lactic acid $\frac{clostridial}{spores}$ butyric acid +CO_2gas+H_2 gas.

When added to milk, sodium nitrate will react with xanthine oxidase to generate sodium nitrite. Nitrites have the ability to inhibit clostridia, reducing one cause of late Because cheese can prevent such a prevalent problem, then why haven't you seen it before, is probably what most of you reading this are wondering. The truth is that meat can contain sodium nitrate as a preservative due to FDA approval. but not cheese!

Nitrate is changed to nitrite by the enzyme xanthin oxidase [4].

NO_3^- $\frac{xanthin}{oxidase}$ NO_2^-.

.



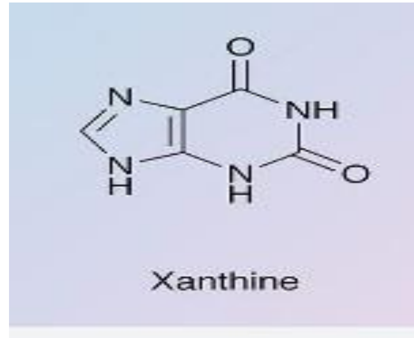


Figure (3) Structure of xanthine

1.4. reasons why nitrates are bad for you

Instead of being broken down by stomach acid, nitrates can be converted into nitrite by your body's microbiome. This can lead to health issues like a higher risk of cancer.

Nitrate salts is an inorganic as sodium nitrate, water -soluble chemical. Although your body produces about 62 mg of nitrates per day, most nitrates are derived from food. An individual takes in 75–100 mg of nitrate daily on average.

Consuming additional nitrates has the following health risks:

- Infants with methemoglobinemia (blue baby syndrome).
- Enhanced susceptibility to cancer.
- Problems that arise during pregnancy.

Because they are stable and unlikely to alter and cause harm, nitrates are comparatively inert.

But they can be changed into potentially dangerous nitrites by the body's enzymes or oral microbes [3].

Moreover, the nitrite presented in stomach as a result of nitrate -nitrite contained food intake, may react with secondary and tertiary amines and amides to form N-nitroso compounds (NOCS) include nitrosamines and nitrosamides.

N-nitroso compounds have the ability to cause cancer. Additionally, nitrosation can happen when food products are being stored or are ripening. Nitrosamines were discovered in cheeses even in the absence of nitrate addition [3].

Nitrites can then convert to one of the following:

- Nitric oxide, which is good for the body.
- Nitrosamines have the potential to cause harm.

Another, Water naturally contains nitrates as well. In certain places, the application of fertilizer can raise nitrate levels to dangerously high levels for young people.

1.4.1. Carsogenic effect

There are conditions conducive to the formation of nitrosamines, which are associated with the presence of nitrates (which can be converted to nitrites), along with amines and acidic pH, all of which enhance their formation. This is a complex process that can be attributed to microbial, chemical, or enzymatic reactions, and their presence in food products depends on various parameters related to the preparation, processing, and storage of the foods, as well as the exposure to heat or other factors. This has been mentioned in several studies as a chemical reaction known as nitrosation, the stage at which nitrosamines are formed in food. This topic is not directly linked to sodium and potassium nitrate, which are separate substances.

Additionally, haem proteins and smoke constituents can react with nitrate when it is transformed to nitrite (Walters, 1992). Acids, phenols, and carbonyls are the primary constituents of the woodwood smoke and liquid smoke utilized in the manufacturing of meat and other food products (Simko, 2011), along with other substances including formaldehyde (Sen et al., 1986). When this molecule and other aldehydes condense with cysteine, nitrosamines are produced. Phenols, however, might stop the nitrosamine from forming. It has been found that smoked food has higher quantities of the non-volatile N-nitrosothiazolidine-4-carboxylic acid (NTCA) than non-smoked food (Sen et al., 1986; Tricker and Kubacki, 1992). Smoking does not seem to have an impact on the levels of the volatile nitrosamines N-nitrosopyrrolidine (NPYR) and N-nitroso dimethylamine (NDMA). When liquid smoke is added to food products, it creates an acidic and antioxidant environment that promotes the conversion of nitrite to nitrous acid, which lowers the nitrite level by forming red nitrosomyoglobin (Girard, 1991). According to Theiler et al. (1984), liquid smoke decreased the volatile nitrosamine NPYR in nitrite-cured fried minced pork flavoring.

Synthesis of nitrosamine in food:

$$N_2O_3 \longrightarrow NO^+ + NO_2^-$$

$$NO^+ + RR'NH \longrightarrow RR'NNO + H^+$$

1.4.2 Methemoglobinemia

Human nitrite reductase is the bacterial enzyme found in the mouth cavity that converts ingested nitrate to nitrite. According to Zeman et al. (2002), nitrate-reducing bacteria may proliferate in newborns due to their stomach's greater pH (2–5) than that of adults (pH 1-3). This may make newborns more susceptible to the growth of methemoglobin. Methemoglobinemia can result from the reduction of Fe^{2+} in hemoglobin to Fe^{3+} by nitrite. In humans, methemoglobinemia is the most common adverse consequence associated with nitrate exposure. Cytochrome B5 reductase has the ability to change Fe^{3+} back into Fe^{2+}. People with cytochrome b5 reductase deficiency through genetics, fetuses, and babies whose cytochrome b5 reductase enzyme concentrations were only found to be roughly 60% of those in adult red blood cells (Ross, 1963; Bartos and Desforges, 1966). The special sensitivity of newborns under 16 weeks of age to methemoglobinemia can be accounted for by the 40–50% reduced activity of cytochrome b5 reductase in comparison to adult levels (which catalyzes the conversion of methemoglobin back to hemoglobin; Bartos and Desforges, 1966; Wright et al., 1999.Also, a significant amount of fetal hemoglobin—which has been demonstrated to produce twice as much methemoglobin as adult hemoglobin in vitro—remains in the blood of these infants (Wind and Stern, 1977; WHO, 2007). A literature search between 1951 and 2015 turned in 151 papers, some of which had carports on methemoglobinemia and suicidal attempts. Methemoglobinemia has been observed to occur at a wide range of dosages. According to Mirvish (1991) and Boink et al. (1999), the range of oral fatal dosages for humans is 4 to 50 g and 67 to 833 mg/kg, respectively. Nevertheless, the information was unsuitable for a dose-response relationship analysis. Under the heading of toxicokinetics, experimental data on the generation of methemoglobin have been assessed (e.g., Lambers et al., 2000; published van Velzen et al., 2008). The data, however, were unsuitable for BMD modeling.

The following reaction occurs when nitrous acid reacts with the sulfhydryl groups in dietary proteins, releasing nitric oxide in an oxidation-reduction process that ends in a disulfide (Pegg and Shahidi, 2004) [6].

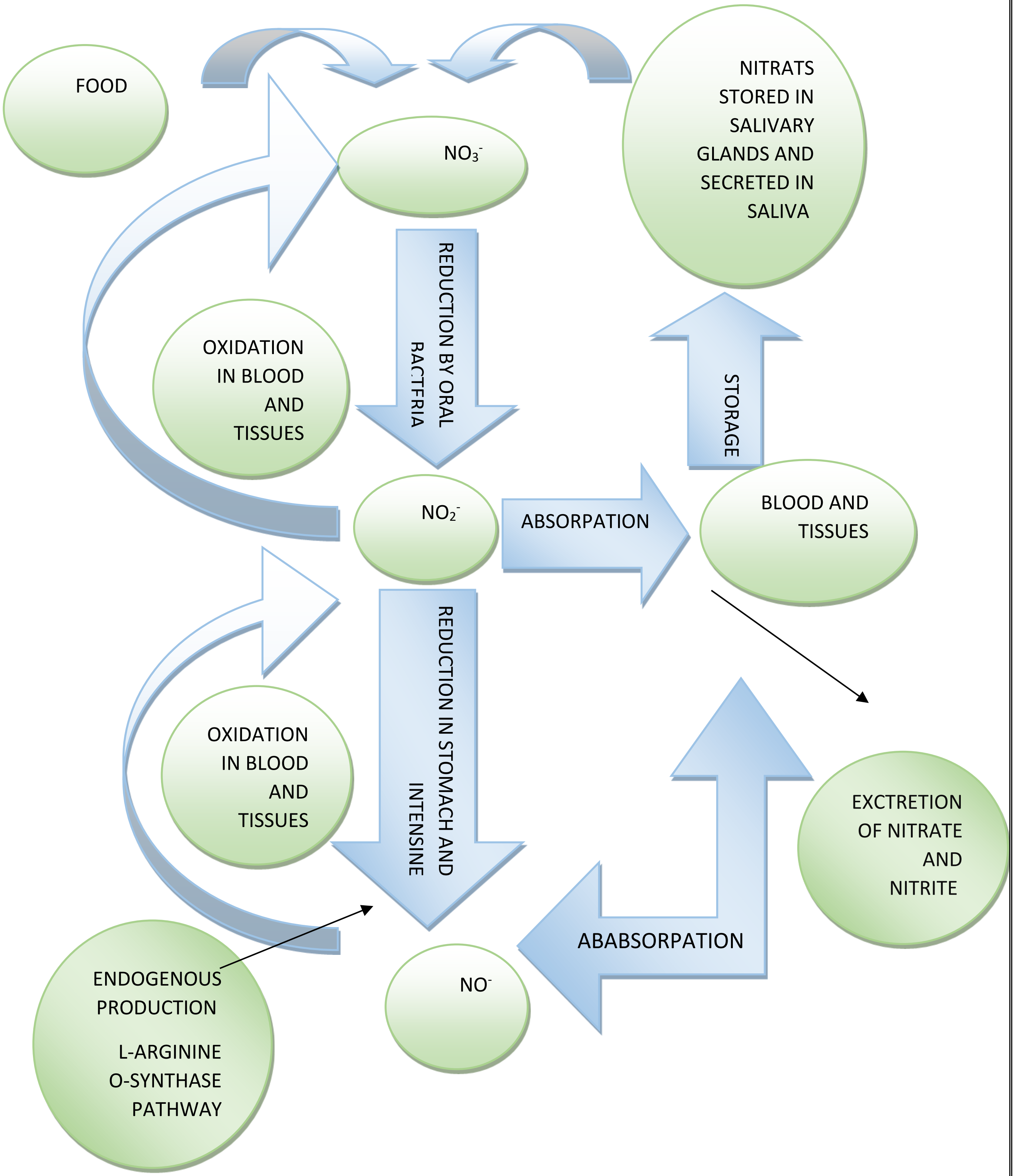


Figure (4)-simplified diagram showing how the body uses nitrite and nitric oxide[7]

1. measurement of nitrate

The high concentration of nitrogen soil may lead to the high nitrate levels in edible vegetables with food products upon nitrates and nitrites (food additive grade) and toxic levels of nitrite may be produced by microbial activity in the gastrointestinal tract of the consumer.

Pfeiffer and Smith (1975) were developed a method of using Gas-liquid chromatography for determination of nitrate content in baby food. The ion selective electrode method, demonstrated a good correlation with the AOAC xylenol method, for routine food analysis. Tanaka et al., (1982), published a method for measuring nitrate in vegetable and product food using a direct, sensitive spectrophotometer [11].

In this further research (figure5), several nitrate determination methods produced during the previous 15 years were examined. The basic principles and analytical parameters of each method, including matrix, detection limits, and detection range, were listed. the benefits and drawbacks of different approaches were assessed [6].

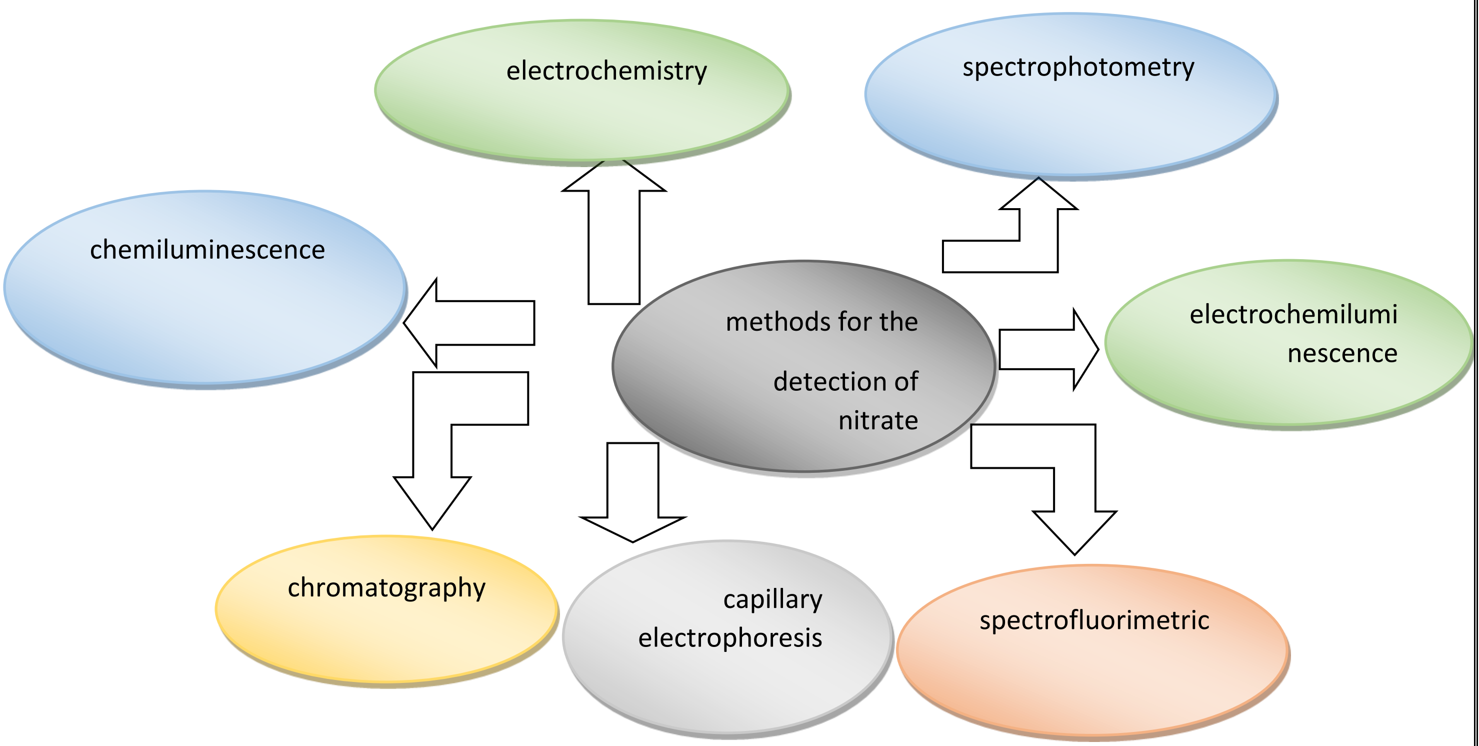


Figure (5)- Different Methods for Nitrate Determination Developed Over the Last 15 Years[7]

1.6.1. Electrochemical Techniques

Over the past few years, a variety of review papers has been published regarding nitrate and nitrite electrochemical detection methods *(Wang et al. 2017, Eshrat et al. 2018).* Various methods and analytical techniques have been presented, among which the voltamperometric method seems to have drawn the most interest. There has been a significant advancement in the application of novel materials and solutions with this technology, which has undergone substantial development. Previous usage of conventional unprotected electrode materials (copper, nickel, platinum, cadmium, gold, and glassy carbon) resulted in issues with their toxicity and passivation (Wang et al. 2017). Using both inorganic and organic catalysts and enzymes to alter an electrode's surface has been one attempt to overcome these challenges. This entails improving electrode surfaces electrolytically to produce extremely focused catalytic activity for redox processes. The development of an active and developed electrode surface improves the kinetics of electron transfer as well as the electrode's sensitivity and response signal.

In order to increase the sensitivity and accuracy of nitrate determination, recent studies have reported the use of magnetic nanoparticles (MNP), bovine serum albumin-coated gold nanoparticles (BSA-AuNP; Shankar, 2018), and magnetic nanocomposite NP-SiO2 (Ispas, 2018) as modifiers in electrochemical sensors. In a nitrite determination assay, a carbon paste electrode (CPE) modified with silver nanotubes (Ag/HNT/MoS2) was utilized (Ghanei-Motlagh, Taher 2018). The strong electrocatalytic nitrite oxidation activity of a graphene composite electrode (GCE) coated with nanoclaster (Ag/Cu/MWNT) was observed (Yi Zhang 2018). A sizable portion of the active surface could be utilized for efficient nitrite detection thanks to nitrogen-doped graphene quantum dots modified with nitrogen-doped carbon nanofiber composite (NGQDs@ NCNF) (Li et al. 2017). When applied to the nanoelectrode surface, nanotubes (Kuralay et al. 2015, Bagheri et al. 2017) and nanocomposites (Rajalakshmi, John 2015, Boussema et al. 2016, Zhang et al. 2016) successfully increased analytical performance nanocomposite. The electrocatalytic potential of nanocomposite materials varies with their composition. Higher sensitivity and better selectivity are provided by nanocomposites made by combining different nanomaterials. displays specific nanoelectrodes together with their detection ranges and restrictions. The remarkable characteristics of nanoelectrodes provide broad detection ranges and low detection levels. According to the study's findings, using nanomaterials in electrode modification has emerged as a viable option. The examination of a variety of environmental samples has demonstrated the immense potential of nanoelectrodes. When compared to traditional approaches, electrochemical methods of nitrite detection offer great selectivity and sensitivity, as well as being quick and affordable (Yi Zhanga et al. 2018)[6] .

1.6.2. Spectrophotometric Techniques

Spectrophotometric methods, most frequently based on the Griess method, which are widely used in the determination of nitrates and nitrites. The Griess reaction, first described in 1879, was initially used to identify nitrites in saliva, and this technique served throughout the following century for nitrite identification in biological fluids. To determine nitrates, prior to the diazotation reaction, chemical or enzymatic reduction of nitrates to nitrites is performed. The two-phase process involves diazotation, where nitrites react with sulphanilamide, and then conjugation, where the diazotation product in an acidic medium reacts with amines, e.g., with N-(1-naphthyl) ethylenediamine (NED). to produce pink azo dye (λ=540 nm). Nitroaniline and p-aminoacetofenon were also used in the diazotation reaction, while 1-naphtol, 1-amino-naphtalen,1-naphtolo-4-sulphonian,1,3-diaminobenzene were also utilised in the conjugation reaction. The key phase in nitrate determination is the reduction to nitrites, which is catalysed by cadmium and copper (Wang 1998), hydrazine with copper catalyser, zinc column (Elis et al. 2011, Merino et al. 2000), titanium or VCl3 (Miranda 2001, Woollard, Indyk 2014, Lin et al. 2016). Biological reduction, which does not produce toxic waste, is sometimes used. Enzymatic biocatalysers employed in the colorimetric reaction includes NaR enzymes or NaR-containing microorganisms (Jobgen et al. 2007, Wittbrodt et al. 2015) *[6]*.

The sensitivity of spectrophotometric detection, a rather straightforward way of determining nitrate and nitrite, ranges from 0.02 to 2 Molary when utilizing the Griess reaction. All nitrates and nitrites can be separately determined using the colorimetric approach. This approach has some disadvantages even though it is straightforward and has relatively low detection limits, interference of some organic substances and some ions, and masking agents such as EDTA (Narayana, Sunil 2009) or trisodium citrate. Although it is frequently and willingly used as a result of the simplicity of measurements and low analytical costs, given that nitrate is detected at a micromolar level, this approach cannot guarantee adequate sensitivity. Complex analytical methods are accustomed to solve such problems, improve precision and decrease the nitrate and nitrite detection limits (*Croitoru 2012)* developed a HPLC/VIS technique for detecting nitrate and nitrites simultaneously in biological samples in the Griess reaction. (*Kunduru et al - 2017)* produced film from synthesized polymers with side chains from phosphoric acid and zinc. These communion nitrate and sodium nitrate at a detection limit of 4 ppm. (*Ibrahim et al, 2019*) proposed a simple and effective method of colorimetric detection using the response of conjugation of diazo p-aminobenzoic acid (PABA)and fluorglucynol in an auric medium, where yellow azo dye was obtained, with an anticipated maximum absorption of 434nm [6].

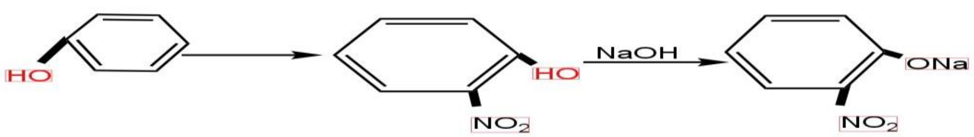
The determination of nitrate as nitrate-nitrogen via digestion becomes difficult. Using an ion selective electrode, Pfeiffer and Smith (1975) measured the nitrate in infant food and found a strong connection with the AOAC xylenol technique. According to Tanaka et al. (1982), some of the techniques used are based on the nitration of bi-substituted phenol, such as 2,4-, 3,4-, and 2,6-xylenol. The 2,4-xylenol approach requires a distillation step, which makes it time-consuming and inappropriate for regular food analysis (Tanaka et al., 1982).

As a result, employed in the creation of a method to quantify the quantity of nitrate in cheese samples using phenol as the active reagent. The principle It was established on the nitration of phenol and the building up the corresponding sodium salt [8].

The elementary processes involved are

$NO_3^- + H_2SO_4 \rightarrow HSO_4^- + HNO_3$ ………… (1)

$HNO_3 + H_2SO_4 \rightarrow NO_2^+ + {}^-OSO_3H + H_2O$ ……… (2)



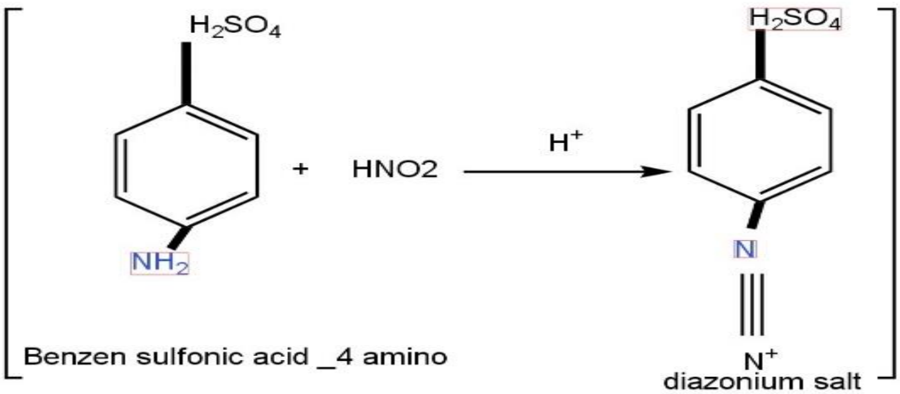
1.7. History Of Azo Dyes

The history of dyeing can be split into two major periods: the "pre-aniline" period, which ended in1856, and the "post-aniline" period, which began in 1856. The former was distinguished by a very small spectrum of colors based on 20 plants and animals that produced dye. The two primary vegetable dyes that were extracted from madder root (from Asia and Europe), which produced a beautiful red color, and indigo plant leaves (from India), which produced the blue dye that is still used in jeans today. One of the most significant animal-based dyes is "Tyrian purple," a costly and well-known color derived from the tiny mollusk nurez. According to ancient legend, this dye was extraordinarily Gorgeous, however data from an old sample shows that it only goes through a drab spectrum of reds and purples. In 1518, a far more exquisite natural color called cochineal, derived from microscopic lice that inhabit specific kinds of cacti, was brought to Europe from Mexico [9].

1.8. Synthesis of azo dye

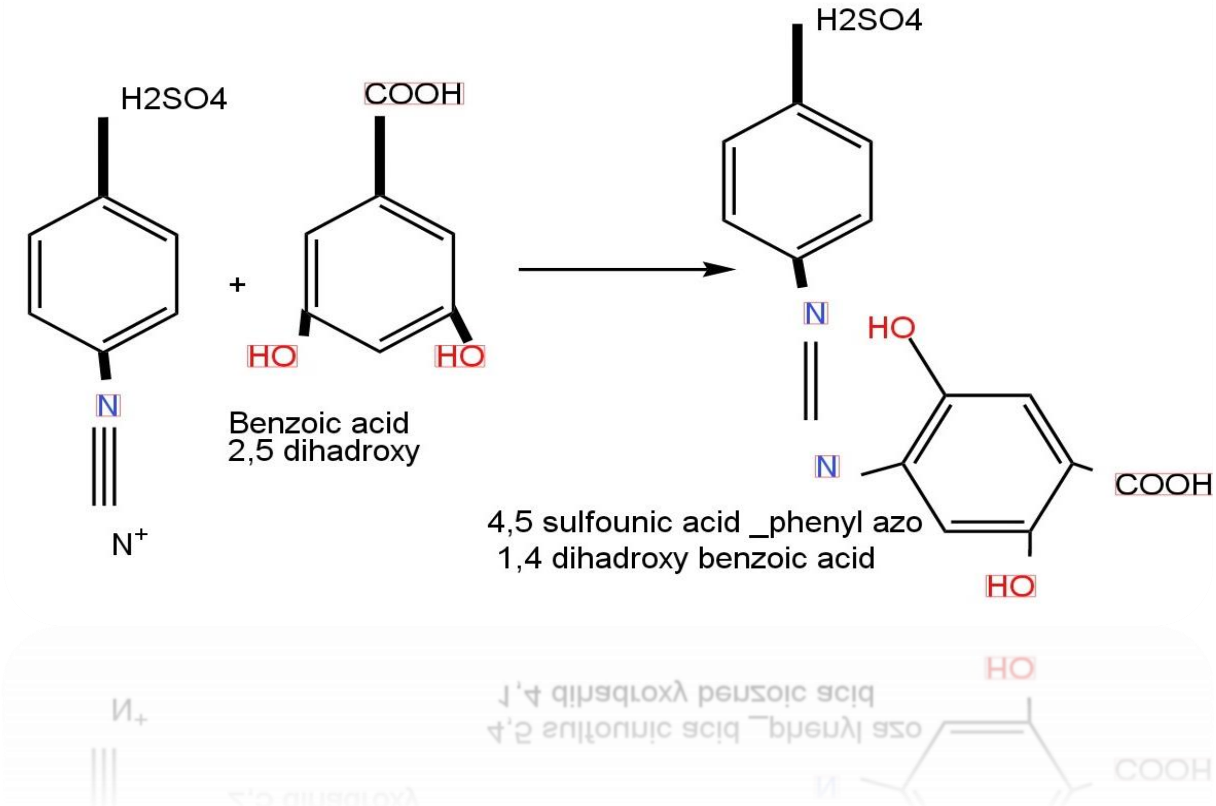
In today's studies, there are working with a class of compounds known as azo dyes, which are molecules that have two aromatic fragments joined by a double bond (N=N). These compounds are commonly used as pH indicators in chemistry investigations. Among the examples are methyl orange and methyl yellow. They are of industrial relevance and are simple to create. The synthesis of an aromatic molecule is the first stage in the two-step reaction that creates azo dyes.

As a result, azo dye is prepared in a two steps reaction. First step, reduction of nitrate by cadmium, then the synthesis of a diazonium salt produced by the reaction of nitrite with benzene sulfonic acid -4 amino (shown below):



disotisation step

second step , the synthesis of an azo dye by the reaction of diazonium of salt with benzoic acid 2,5 dihydroxy (shown below):



Literature review

2.1. Literature Review

Despite The reality that all different kinds of cheese are considered to be a part of the important sources of food for humans, it may not be free from being a source of harmful elements, such as microbial contaminants, and the presence of different amounts of nitrate and nitrite. The national academy of sciences (NAS) stated that vegetables provide 87% of nitrate in a normal diet, while the other products including fresh and cured meat and dairy provide the remainder [1]. However, nitrate and nitrite as the sodium or potassium salts have also been used as food additives in cured meats for many years [6].

Toxic rate of nitrite and nitrate maybe produced by microbial activity in the bod (gastrointestinal tract) of the consumer (*tanak .et al 1982).* Nitrate containing raw milk such as are potential causes of methemoglobinemia a disease which may occur in children older than 6months *(Sanchez-Echaniz et al,2001).* The determination of nitrate most frequently based on the reduction of nitrate to nitrite (page 2). Then Griss method or chromatography with conductivity detection and another electro methods. [10,11]

There are multiple studies based on the Griss method using the Spectrophotometer device different from the reagents, but they gave close results with a slight variation, the analysis obtained results reveal a variation of nitrate level between 0.87-17.52mg\kg *(l.tudoand et al2007)* in Romania different types of row milk (cow and sheep row milk) highest level of nitrate were obtained, but *(Ali Topcu and anther 2005)* the results shows that nitrate level was found between (0.92 -22.40) with mean (8.96±4.93) mg\kg. In Turkish white cheese produced from cow's milk, sheep's milk found to be between (0.47-23.68) (with mean±6.28 mg\kg). *Mohammed Amer Zamirk 2013* result for determination of nitrate in cow's and sheep's milk cheese reveal an average of 5.10 and 6.25 mg /kg respectively [12,13,14].

Different methods to detection of nitrate depended on sensitivity, variation, selectivity, low limits of measurement, low cost and its facility availability.

Are all methods passed the analyzes? the comparability is important to detection of nitrate in 1995 collected *(cathy and another)* twenty forage samples for variation in nitrate, each forage sample was analyzed 4 times by 4 different methods: diphenylamine spot plate, spectrophotometric, nitrate -selective electrode and high – performance liquid chromatographic. five feed extracts were spiked with 2 different amounts of nitrate and analyzed by each method. The percentage recoveries for the spectrophotometric and nitrate-selective electrode methods were nearly 100%, while the nitrate-selective electrode method had the least variation and was the least accurate. The variation in the nitrate -selective electrode method was lower ($p>0.05$) than the other methods, the average coefficients of variation for all samples within a method were 15% ,12%,6.4% and 16% for diphenylamine spot plate, spectrophotometric, nitrate -selective electrode, and high-performance liquid chromatographic methods, respectively. So, it's been highlighted and suggested that the nitrate -selective electrode methods is more accurate and precise than the other method of analysis tested [15].

The average percent recoveries for the spectrophotometric and considered a rough estimate of the nitrate level. Nitrate -selective electrode methods were closer to 100% than the other methods, which were greater than 100%. The spectrophotometric and Nitrate -selective electrode methods also had similar SD of recovery, suggesting that they may be better methods for estimating nitrate levels in forage [15].

The new recent work concerning nitrate measurement has embraced the classical reagents several reported spectrophotometric methods involve the use of common reactions, such as a reduction reaction followed by diazotization, nitration reactions, or others. The well_ know spectrophotometric methods for the detection of nitrate are based on the nitration of phenolic compounds ,2,4 xylenol, 2,6 xylenol (*Tanaka et al., 1982)* and *chromophoric* acid *(*Basset *et al. 1978)* and another reagent. *Gaya UI and Alimi* in 2006 described rapid and sensitive spectrophotometric method for determination of nitrate in vegetables. The method is based on the detection of the absorbance of yellow sodium nitro phenoxide formed via the reaction of phenol with the vegetable-based nitrate in presence of sulphuric acid. The amount of nitrate found is significantly impacted by the acid's analytical concentration. Overnight, the color developed quickly and stayed consistent. Analysis of selected vegetable samples containing nitrate gave satisfactory mean recoveries of 76 to 123% in 18 detections. The proposed method is reproducible and sensitive to less level concentrations, so *Esasu Gebne and another in 2018* used same method to detection of nitrate in some kind of vegetables, but nitrite was determined by forming violet AZO dye while nitrate indirectly by phenol to forming yellow sodium nitro phenoxide. The discuss of the study showed that the nitrite content in vegetables was rate from (0.46 -1.44) mg\kg. nitrate content was also rate from (28.74 – 230.5) mg\kg.

The highest nitrate or nitrite concentration was detected in cabbage followed by anion and tomato. The different sampling areas showed no signification effect at (P=0.05) on the nitrate and nitrite contents in same vegetables tested in this study. However, the levels of nitrate and nitrite were significantly different (P=0.05) among vegetables. The nitrate and nitrite levels generally were below the allowable range set by World Health Organization (WHO) [18,19,20].

Sajad Chamaudust, Mohammed Reza and another in 2016 were developed a new method for the simultaneous determination of nitrate and nitrite in milk samples using by ion chromatography proposed mobile phase composed of sodium hydrogen carbonate and sodium carbonate (1.0 and 3.3 mmol\L) with a flow rate of 0.7 ml\min. the average recoveries for nitrate and nitrite were higher than 86%and 88% respectively. The detection limit of detection for nitrate and nitrite were 0.24 and 0.09 mg\L respectively. The results of 102 samples (real nulk) showed nitrate was found in all of the samples 100% with a mean of 34±11 mg\L, the mean intake of nitrate in all age groups was lower than World Health Organization (WHO) guideline [21].

The content of nitrate and nitrite ions in homogenized meat samples of baby foods was measured by a validated method based on ion chromatography (IC) coupled with conductivity detection in 2020 for the first time in Donatella Coviello and another. The results showed that nitrate contents were below the European (EU) fixed value of 200 mg.kg-1.[22]

Finally, determination of nitrate in stage makes cheese and dynamics of nitrate during the make cheese, however *B Korenekova in 2000* was to observe the dynamics of nitrates and nitrites during the six stages of manufacture of Emmental cheeses. Samples were taken of untreated milk, of pasteurized milk of milk with nitrates added, of pressed cheese cured, of whey, of maturing cheese and of the final product, the samples were drawn from a commercial operation in a cheese factory in the eastern part of Slovakia. The mean $NaNO_2$ content in untreated and in pasteurized milk was 0.2 and 0.1 mg\kg respectively and the mean $NaNO_3$ content was 0.9 and

0.9 mg\kg respectively. Nitrates were added to the milk to stop microorganisms from blowing hard cheese. The mean concentration of milk containing nitrate was 81.2 mg/kg, with a maximum value of 90.0 mg/kg. Following a press. It was discovered that the average nitrate levels was 20.6 mg/kg $NaNO_3$.

A considerable quantity of nitrates passed into the whey. Where the mean nitrate content was 67.0mg\kg $NaNO_3$.

The final product had a markedly lest content of nitrates (3.3 mg\kg) $NaNO_3$ when compared with the values in cheese during maturation (11.3 mg\kg $NaNO_3$) [23].

Susan Ganualdi and another in 2018 said nitrates and nitrites can be present in dairy products from both endogenous and exogenous sources. In the European Union(EU) ,150 mg\kg of nitrates are allowed to be added to the cheese milk through the manufacturing process .The CODEX general standard for food additives has a maximum permitted level of 50 mg\kg residue in cheese , In order to examine imported cheeses for nitrates purposefully added as preservatives and the endogenous quantities of nitrates and nitrites prevalent in cheese in the United States, nitrates 4 are not authorized for use as food additives in cheese in the U.S. Marketplace, a method was advanced and validated using ion chromatography with conductivity detection .
A market sampling of cheese samples purchased In the Washington DC metro area was performed. in 64 samples of cheese, concentrations ranged from below the method detection limit (MDL) to 26 mg\ kg for nitrates and no concentrations of nitrites were found in any of the cheese samples above the (MDL) of 0.1 mg\kg. A highly of the samples (93%) had concentrations below 10mg\kg, which indicate the presence of endogenous nitrates, the samples with concentration s above 10 mg\kg were mainly processed cheese spread, which can contain additional ingredients often of plant-based origin these ingredients are likely the cause of the elevated nitrate concentrations. The analysis of 12 additional cheese samples that are liable to the intentional addition of nitrates ,9 of which were imported, indicated that in this limited study concentrations of nitrate in the U.S- produced cheeses did not different those in imported samples [24].

Objectives of the study:

1. Five steps were involved in the preparation of the cheese samples: grinding, weighing, dissolving in warm water, precipitating the fat and proteins, and filtering.
2. The extraction and determination procedures were depended on the Griess method,

Prepare a series of standard nitrate solutions (ppm) by diluting the sodium nitrate, then measure the absorption of solution, draw the standard curve that represents the relationship between absorption and concentration.

3. Determine prepared samples using by spectrophotometric, compare external addition and samples to know concentration of samples.

4. Is the nitration method suitable for determining nitrates in cheese as a type of food consumed daily, compared to the discuss of the approved method for determining nitrates?

In summary, while the nitration method can be used for determining nitrates in cheese, its suitability compared to the approved method depends on several factors. It is advisable to consult relevant regulation and scientific literature, and conduct validation studies to ensure the accuracy and reliability of the discuss.

Experimental

3.1. Experimental

3.1. Chemical material:

All chemical used were of analar grade and used as received from the company without further purifications E. Merck KG Darmstadt-company.

Sodium Nitrate. $NaNO_3$. Sodium Hydroxide. NaOH. Zinc Sulphate (heptahydrate).

ZnSO4.7 H2O. Sulphuric Acid. H_2SO_4. Phenol (Carbolic Acid). C_6H_5OH. Silver Sulphate.

$AgSO_4$. Sodium Carbonate. Na_2CO_3.

3.2**. devices used:**

- Electric Balance

- Drying Oven: memmert

- Hot Plate Stirrer: tyb: PZ60

s/No:3810208

volt: 230- watt:2000

Harrygestigkeit Gmbh

- Water bath: memmert

- Spectrophotometric: DR3900

- Ultra-bath soundtybe: SWB20

DIN:12876

Klasse:1

Nr:7370029Germany

3.3. Preparation of material

- Sodium Nitrate ($NaNO_3$): was prepared by dissolving 0.1371g sodium nitrate (which was dried in drying oven at 70c° for24h) in water and diluting to 100ml. Working standard solutions (2-4-6-8-10) mg. l^{-1} were prepared by appropriate dilution of stock solution daily. By volume of stock solution (0.02-0.04-0.06-0.08-0.1) ml selectively.
- Sodium Hydroxide2% (NaOH): was prepared by dissolving 2g sodium hydroxide in water and diluting to 100 ml.
- Zinc Sulphate heptahydrate 12%($ZnSO_4.7H_2O$): was prepared by dissolving 12 g zinc sulphate heptahydrate in water and diluting to 100ml.

- Phenol solution 8%(C_6H_5OH): was prepared by dissolving 8g 0f phenol in water diluting to 100ml.
- Silver Sulphate ($AgSO_4$): was prepared by dissolving 5g silver sulphate in 7ml of sulphuric acid then add water diluting to 100ml.
- Sodium Carbonate (Na_2CO_3): was prepared by dissolving 10g of sodium carbonate in water diluting to 100ml.
- Sulphuric Acid (H_2SO_4): concentration 98%, density 1.84 mg/cm^3.

3.4. Samples Collection

The 39 samples of Libyan imported and local white cheese have been analyzed; the samples were produced from cow's milk. Samples imported were obtained randomly from Tripoli local and open markets in Libya. All samples were kept at +4˚C. Analysis was conducted during days of bringing the samples. Samples were collected and analyzed in the period of time between November 2020 to September 2021.

3.1.5. Method of Analysis

The extraction and determination procedures were based on the Griess method, The preparation of samples passed through five stages starting from grinding, weighing, dissolution in warm water, precipitation of the fat and proteins, ending with filtration. The nitrate in a portion of filtrate was reduced to nitrite by means of copperized cadmium. For the determination of nitrate in the reducing form (nitrite), then synthesis of a diazonium salt by reaction nitrite with benzene sulfonic acid -4 amino, and synthesis of an azo dye by reaction diazonium salt with benzoic acid 2,5 dihydroxy, were used as coloring developing reagents. Measurement of the color intensity was made by spectrophotometer at a wavelength of 400 nm, and compared with standard nitrate solutions. [29].

3.1.5.1. First Method

- 8g of cheese sample was taken and dissolved in150ml warm water at a temperature ranging (50-70 c°) and mixed thoroughly to ensure no cheese residue remains. Sometimes a water bath is needed to achieve complete dissolution.
- 10ml of 12% $ZnSO_4$ and 10 ml NaOH 2% was added to sample, and the mixture was stirred. After 10 minutes ,100 ml of cold water was added, and the mixture was filtered. then, a nitrate Absorbance was measured using a device at λmax =400 nm.
- Finally, we compared the absorbance of the samples with the standard solutions to determine the concentration of the sample [14,29].

3.1.5.2. Second Method

8g of cheese sample was taken and dissolved in150ml warm water at a temperature ranging (50-70 c°) and mixed thoroughly to ensure no cheese residue remains. Sometimes a water bath is needed to achieve complete dissolution.

10ml of 12% $ZnSO_4$ and 10 ml NaOH 2% was added to sample, and the mixture was stirred. After 10 minutes ,100 ml of cold water was added, and the mixture was filtered.

4ml of sample was taken into a test tube cooled in ice of prepared sample. 1 ml of 5% Ag_2SO_4 solution was added followed by subsequent addition of 7 ml of 98% H_2SO_4 and 0.1 ml of 8% phenol solution. The solution was allowed to stand for 20min while shaking occasionally. The resulting mixture was extracted in 50 ml separating funnel by adding toluene -For purifying the sample and obtaining the crystalline sodium phenoxide in the organic phase, as well as improving the separation process in general-, and shaking for 5 to 10min. The lower aqueous layer was discarded. The organic phase was washed twice with 10ml of distilled water and Shaked for 2min in each time the aqueous phase was discarded. The organic phase was extracted again by shaking for 1 min with 10 ml of 10% Na_2CO_3 solution

and collected in a test tube. Absorbance was measured at λmax=400nm [18].

During the organic phase separation, sodium carbonate (Na_2CO_3) is added for several reasons

- Phase cleaning: Sodium carbonate acts as a strong cleaning agent in the organic separation process. It is capable of removing organic substances adhered to the phase and cleaning it effectively.
- pH adjustment: Sodium carbonate can modify the acidity level in the organic phase, which helps improve the efficiency of the organic separation.
- Reaction with certain impurities: Sometimes, sodium carbonate can react with some organic pollutants or impurities to facilitate the separation process.

In general, the addition of sodium carbonate during the organic phase separation process is Considered necessary for cleaning and improving the efficiency of the process [18].

4.1. Results

Nitrate was determined in all cheese samples with a total of 39 samples of Libyan imported local white cheese ,9 of the samples were produced from spread cheese, the 13 samples were produced from cream cheese ,6 samples were produced from mozzarella cheeses,3 samples were produced from feeta cheeses and 8 samples were produced from semi soft cheeses. Results for nitrate of all samples were presented separately in Table 1,2,3,4,5. Nitrate content was found to be between 3.49 mg/kg to 174.20mg/kg in all types of cheese samples with 6.07 mg/kg as an average. The nitrate content in spread cheese was found to be between 7.00 - 37.93 mg/kg with 15.11 mg/kg as an average, while the nitrate content in cream cheese was found to be between 20.46 mg/kg to 174.20 mg/kg with 48.32 as an average ,the nitrate content in mozzarella cheese was found between 3.49mg/kg to 40.18 mg/kg with 23.17mg/kg as an average ,but nitrate content in feeta cheese was found to be the highest concentrations between 151.24 mg/kg to 171.20 mg/kg with 158.22mg/kg as an average and nitrate content in semi soft cheese was found between 20.59mg/kg to 50.16 mg/kg with 33.55 mg/kg as an average .

4.1.1 Calibration curve for first method:

Sodium Nitrate ($NaNO_3$): was prepared by dissolving 0.1371g sodium nitrate (which was dried in drying oven at 70c° for24h) in water and diluting to 100ml working standard solutions were prepared by appropriate dilution of stock solution daily :(2-4-6-8-10) mg. l^{-1} by volume of stock solution (0.02-0.04-0.06-0.08-0.1) ml selectively

Standard concentration (mg/l)	**Absorption**
0	0.00
2	0.177
4	0.385
6	0.527
8	0.719
10	0.941

Table 2. absorption and standard concentration of nitrate

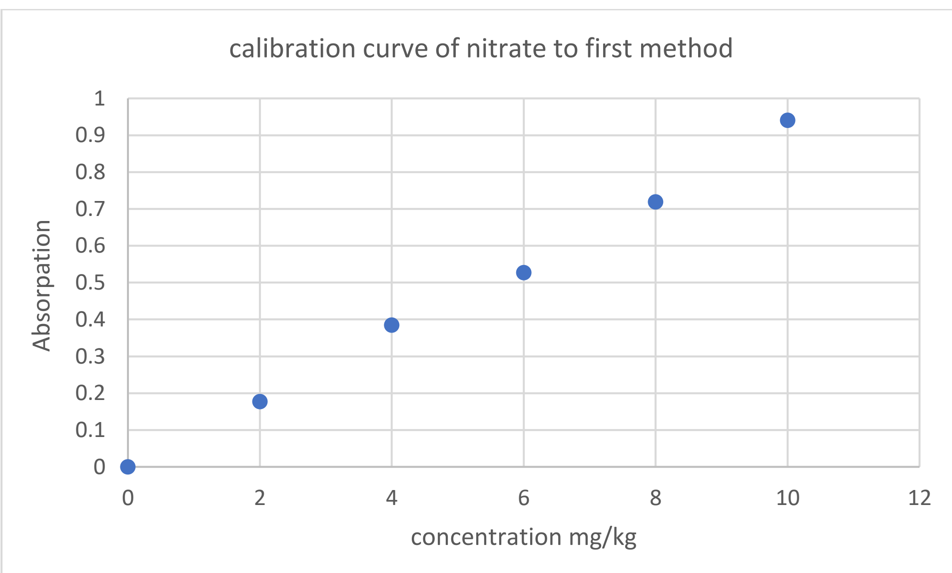


Figure (9). calibration curve for nitrate

4.1.2. Kind of cheese

1-Spread cheese

Concentration of NO_3 mg/kg	**Name of cheese**	**Concentration of NO_3 mg/kg**	**Name of cheese**
6.98	Happy cow	19.22	Delice(tringle)
9.40	Landor	13.97	Delice(square)
7.48	Happy cow (namsa)	10.98	Pock
11.48	Master	18.47	Kiri
37.93	Aboalwalled		

Table 3. concentration of nitrate in spread cheese.

Figure 10-concentration of nitrate in spread cheese

2-cream cheese

Concentration of NO_3 mg/kg	**Name of cheese**	**Concentration of NO_3 mg/kg**	**Name of cheese**
27.45	Azahrat	24.71	Philadelphia light
20.46	Philadelphia	52.77	Cream cheese natural
34.94	Halibi	34.65	Alawafy
23.71	Happy cow cream	59.61	Puck olives
75.87	Elle and vize (France)	37.68	Puck less fat
174.20	Amaray demasheg	27.95	Master cream
34.10	Recotta cheese (ready)		

Table4 concentration of nitrate in cream cheese.

Figure (11)- nitrate concentration mg/kg of cream cheese

3.Mozzerella cheese

Table 5. concentration of nitrate in mozzarella cheese

Concentration of NO_3 mg/kg	**Name of cheese**	**Concentration of NO_3 mg/kg**	**Name of cheese**
38.43	Mozzerella	27.95	Mozzarella Turkish
7.24	Mozzarella (Germany)	21.71	Ile de France briec
40.18	Mozzarella chimney	3.494	Cheddar white(ready)

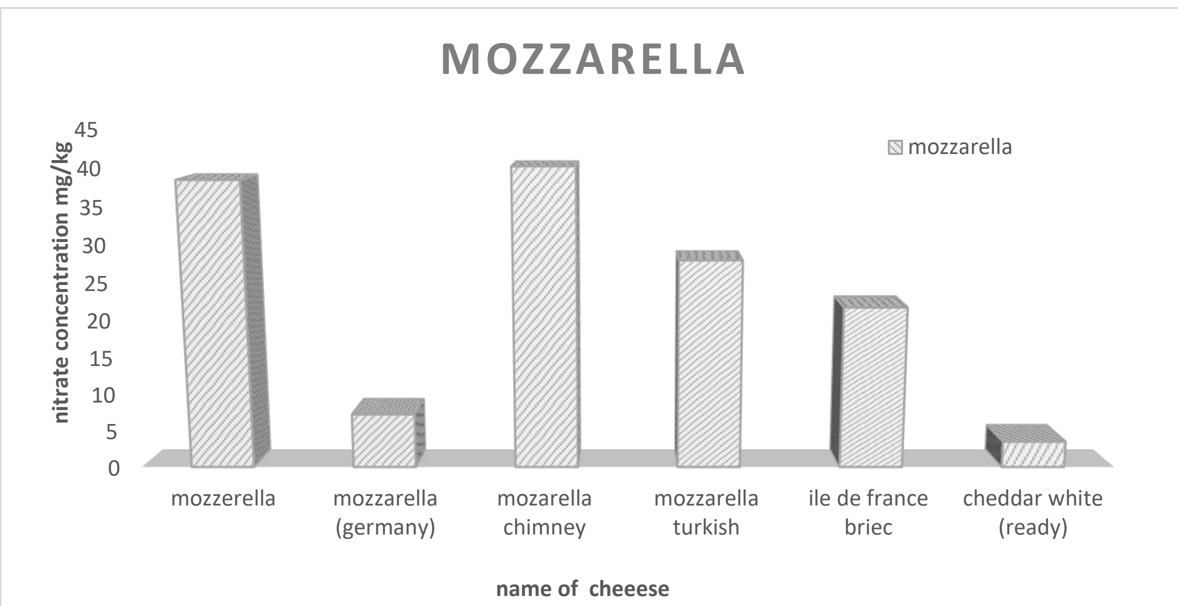


Figure (12)-nitrate concentration mg/kg of mozzarella cheese

4-semi soft cheese

Table6. concentration of nitrate in semisoft cheese.

Concentration of NO_3 mg/kg	Name of cheese	Concentration of NO_3 mg/kg	Name of cheese
41.927	Mental normal	42.36	Kraft
24.451	Mental Germany	50.16	Tebra (slide)
21.997	Squeezed cheese (salty)	45.67	Lactima(slide)
21.21	Happy cow (box)	20.59	Squeezed cheese (normal)

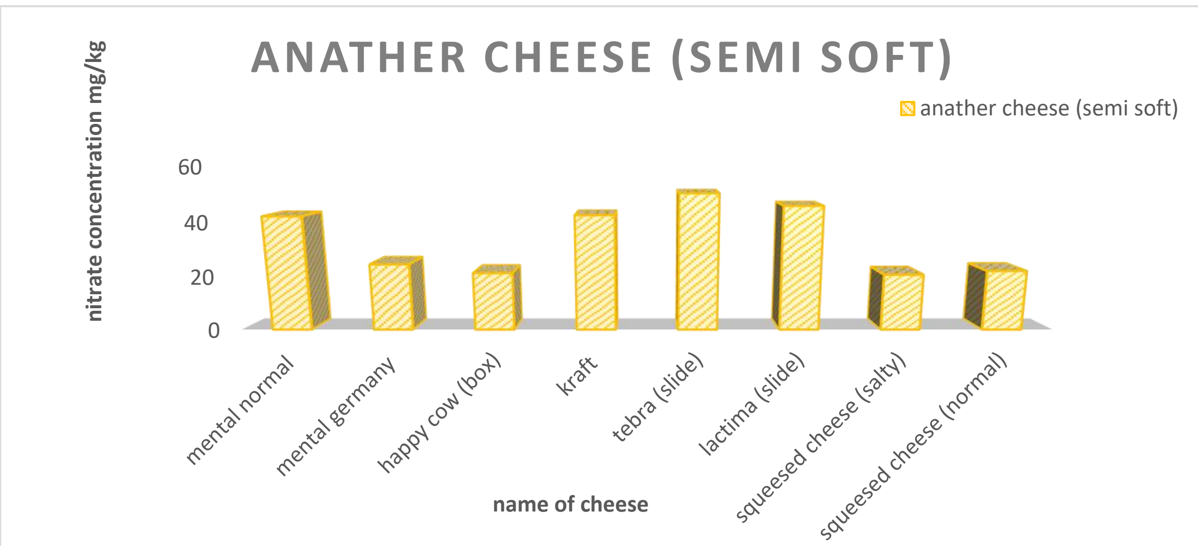


Figure (13)-nitrate concentration mg/kg of semisoft cheese

4-Feeta cheese

Concentration of NO_3 mg/kg	Name of cheese
171.20	Gebnety with chata
152.23	Gebnety with olives
151.24	Grean land (normal)

Table7. concentration of nitrate in feeta cheese.

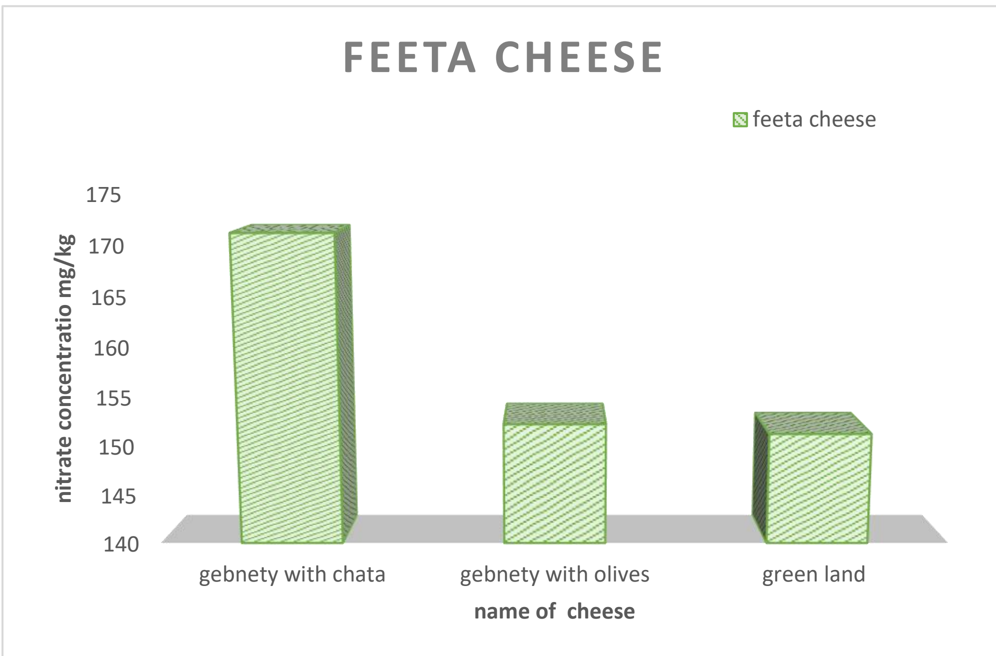


Figure (14)-nitrate concentration mg/kg of feeta cheese

4.2 discussion of first method

In Libya, white cheese is the main type representing the total production of cheese made from cow's milk. Libyan white cheese is produced using traditional methods (small-scale processing facilities in villages). The nitrate levels are similar to those of most countries, and the manufacturing process for white cheese does not differ much, although it varies depending on the type of cheese. The maximum allowable nitrate values according to some countries, in addition to Libya LNCSM, FAO, G.C.C(GSO) and EOS are50mg/kg [27,26]. However, it is important to note that production and safety standards for white cheese in Libya may differ, and it is necessary to ensure that the cheese is produced and handled in a safe and healthy manner to avoid any potential health risks [14,30].

The curve was excellent as it reached an R value of (r^2= 0.997) for the first method, and the following equation was applied to determine the concentration.

Y=0.0925X-0.0042.

Average= $\boldsymbol{\bar{x} = \frac{1}{n}\sum_{i=1} x_i}$

X^{-}: Average.

X_i: represents the individual numbers in the table list.

$\sum_{i=1}$: signifies summing all the numbers in the table list.

There are many different kinds of cheese in the way they are manufactured, but cooked cheese and mozzarella cheese are more common in the Libyan markets, and there are special markets that include various types of cheese in this study after collecting samples from various Tripoli markets, The first method was used to analyze nitrate, (Chapter II Page 24).

The study conducted to estimate the nitrate content in samples of white cheese using spectroscopic analysis to measure the nitrate concentration in the different samples of white cheese most commonly traded in Libyan markets, The results showed that after dividing the cheeses into different types based on their appearance, manufacturing method, and trading in the markets, the nitrate concentration in them varied. Both cooked cheese, creamy cheese, and mozzarella cheese did not exceed a nitrate concentration of 50 mg/kg. However, some types of creamy cheese, feta cheese, and sliced cheese classified under other types of cheeses exceeded this limit, The results by type of cheese were as follows:

4.2.1 Spread cheese

The concentrations ranged between the maximum value and the minimum value (37.93-6.98) mg/kg, and the concentrations were within acceptable limits according to food standards. There were no health risks associated with the nitrate content in this cheese. The average reading for these samples was 15.11mg /kg, The canned cheese Delice squares had the lowest nitrate concentration at 13.97mg /kg compared to the same type canned cheese (Delice tringle) in strips, which had a concentration of 19.22mg /kg. Happy Cow had the lowest nitrate concentration of all creamy or spread types at 6.98 mg/kg, while AbuElwaled had the highest nitrate concentration at 37.93 mg/kg for this type. All concentrations were in compliance with food standards and did not exceed the permissible amount, which is 50 mg/kg [26,27].

4.2.2 Cream cheese

It's fresh, soft cheese, and different from the first type of taste, its creamy cheese, which is not natural, made of milk and cream, which contains 33% milk fat, and the addition of Tactical Acid bacteria to scented and homogenous milk, which causes the liquid to coagulate, gives it the cream of milk and contains more fat than other cowards, Creamy soft cheese samples studied represent the most diverse in Libyan markets. Thirteen samples were taken, and the results showed the highest concentration of nitrates and the lowest concentration ranged between (174.20-20.46) mg/kg, Philadelphia cream cheese had the lowest concentration, while Almaray demasheg cheese had the highest concentration of nitrates, locally manufactured ricotta represents a concentration of 34.10 mg/kg. The olive-flavored Buck cheese showed a higher concentration than regular Buck cheese without any additions. The same results were found for fat-free Philadelphia cheese having a higher concentration than regular Philadelphia. This implies that any addition to the cheese or a change in the original ingredients results in an increase in nitrate concentration, indicating the need to add nitrates to maintain cheese quality.

Elle cheese is a French creamy cheese with a slightly salty taste. The results showed a high concentration, but fortunately did not exceed the nutritional standards. Unfortunately, it is not available in Libyan markets. Almarai cheese exceeded the standards and limits for nitrate concentration, reaching more than 150 mg/kg at 174.20 mg/kg, with an average of 48.32 mg/kg for these readings. These values are far from the data and results of previous studies mentioned in Chapter Two.

4.2.3 mozzarella cheese:

Mozzarella cheese showed the best results and did not exceed the nutritional standards for nitrate concentration. It is one of the most commonly traded cheeses in Libyan markets. The cheese with the lowest concentration was cheddar at 3.49 mg/kg, while the highest concentration of nitrates was in mozzarella chimney at 40.18 mg/kg. This increase in concentration is due to the addition of liquid smoke to the mozzarella cheese, which led to an increase in concentration to maintain cheese quality. The average nitrate concentration for this type was 23.17 mg/kg, which is the best among the studied cheese varieties.

4.2.4 Semi soft cheese:

The other cheeses classified here varied in packaging method between sliced, ready-to-eat, and canned cheeses, totaling 8 types. The results according to food standards and specifications showed that the nitrate concentration did not exceed 50 mg/kg for this type of cheese. The maximum concentration was in Tabra at 50.16 mg/kg, and the minimum was in squeezed ready-to-eat cheese at 20.59 mg/kg. The average readings for this type of cheese showed 33.55 mg/kg.

4.2.5 feeta cheese

Three samples were taken of Feta cheese, which is the least diverse cheese in Libyan markets. The results showed an increase in nitrate concentration exceeding the standard specifications. This increase is due to the excessive saltiness of this type of cheese and other additives such as olives and hot peppers. The average readings were higher than the specified concentration, reaching 158.28 mg/kg, making it the worst cheese and not recommended for consumption for any purpose, leading to its prohibition from trading in Libyan markets.

4.1.2. Calibration curve for second method:

This method, applied for nitrate estimation using Nitration with a spectrophotometer, is not commonly used due to interferences between the sample and other components during absorbance measurement. This method has been implemented on samples of water and vegetables that do not require processing. Nitrates are directly measured without adding specific substances, unlike cheese, which is processed to remove fat and protein to obtain purified nitrates in water (see page 24**) .**This method was applied to 5 samples of cheese, which had previously been subjected to the initial method for comparison of the two methods to determine the accuracy of the first method used (see page 24). The difference between the two methods lies in the fact that the first one relies on Griss, while the second one relies on Nitration, and the latter is not applied to processed samples like cheese. A calibration for nitrates will be performed using the second method:

Sodium Nitrate ($NaNO_3$): was prepared by dissolving 0.1371g sodium nitrate (which was dried in drying oven at 70c° for24h) in water and diluting to 100ml working standard solutions (1-3-5-7-9-11) mg. l^{-1} were prepared by appropriate dilution of stock solution daily by volume of stock solution (0.02-0.04-0.06-0.08-0.1) ml selectively.

Concentration NO_3	**Absorption**
0	**0**
1	**0.01**
3	**0.05**
5	**0.13**
7	**0.15**
9	**0.20**
11	**0.25**

Table8. Absorption of nitrate to standard concentration

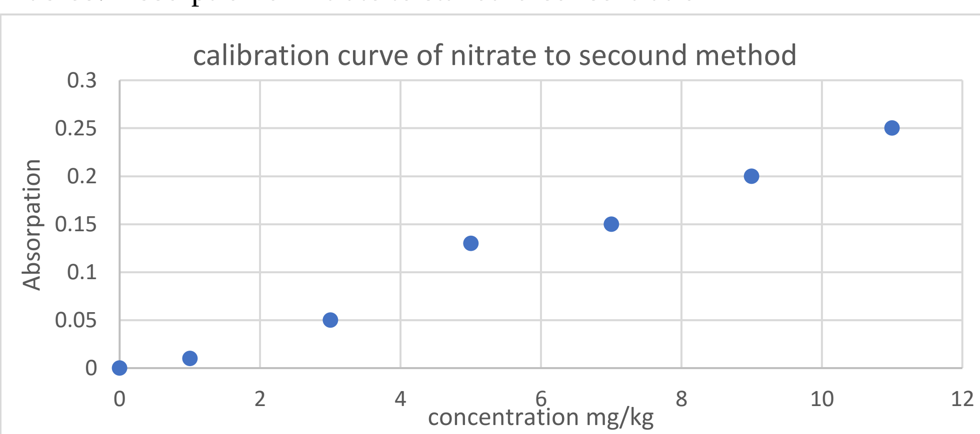


Figure (15)-calibration curve of nitrate of second method

4.1.3 comparative method (1) and (2) to determined nitrate in cheese

The first and second method was used for a set of samples and compared the effectiveness of the second method in estimating nitrate for treated cheese samples.

Concentration of NO_3 (2)	Concentration of NO_3 (1)	Name of cheese
23.98	**21.99**	**Squeezed**
19.73	**11.48**	**Master**
38.61	**37.67**	**Puck light**
79.47	**52.77**	**Cream Germany**
72.47	**151. 24**	**Green land**
73.84	**50.16**	**Tebra**
53.8	**37.93**	**Aboalwalled**

Table 9. comparative second method with first method

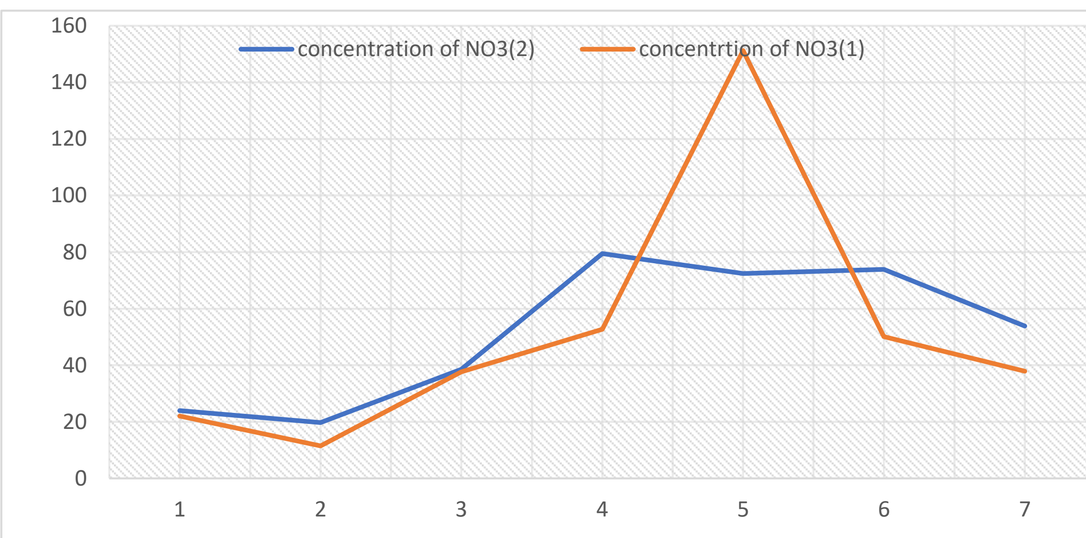


Figure (16)-comparative method (1) and (2) for determination of nitrate in cheese

4.3 Discussion for second method:

The curve was excellent as it reached an R value of (r^2= 0.987) for the second method, and the following equation was applied to determine the concentration.

Y=0.0233X-0.0068.

The second method based on nitrogen was applied to 7 samples previously tested with the first method based on Griss, and the results showed variation and differences in nitrate concentrations. Some samples had a (±2-unit) difference, while others were higher than that**.** During the second method analysis of the samples, those showing a (± 2-unit) difference in concentrations had clear color intensity and stability during absorbance measurement, while the other samples exhibited color that appeared for a period and then disappeared, indicating the presence of interferences leading to instability with sample components. The results also showed that an increase in nitrate concentrations leads to an increase in interferences with sample components. This was evident in the samples of greenland and Tebra where the concentration was higher than 50 mg/kg, and the difference was very significant in terms of increase or decrease. It can be concluded that the second method relying on nitration is not suitable for routine estimation of nitrates as it shows inconsistency in results, interference of components, and significant deviation from data accuracy when compared to the first method r^2=0.639. Because nitro phenoxide has an unstable content, the analytical concentration of nitro phenoxide has a notable impact on the nitrate measured (page13).

Moreover, the observation of elevated nitrate readings could potentially be attributed to the existence of nitrate in raw milk. The main causes are the outdated manufacturing methods and the inability to regulate the hygienic conditions, which might differ from location to location. These include:

• The possibility of unintentional contamination by sources of fertilizers within the plant.

• Using home wastewater and nitrogen fertilizer-related nitrates in technical processes that contaminate water [6].

• Nitrates are purposefully added as a preservative to slow down the potential for bacterial growth, which can be brought on by:

• Hand milking is a common technique [8].

• A high amount of moisture combined with varying salt ratios (white cheeses have a water content of over 55 %) [6].

- Lack of thermization treatment affects the majority of Libyan cheese. In certain instances, the produced cheeses are not kept in an appropriate hygienic environment during handling, storing, and selling in the open market (sidewalk market).

It appears that the aforementioned factors may have had a greater impact on the nitrate concentration of cheese prepared from cow's milk. Less than 50 mg/kg of nitrate was found in spread, semi-soft, mozzarella, and certain cream cheese samples out of all the samples. However, the samples of feeta cheese had the highest nitrate content—more than 150 mg/kg.

Numerous nations have conducted studies on the nitrate levels of various cheese kinds. For example, a study conducted in Turkey by Topçu et al. (2006) found that the nitrate levels of white cheeses ranged from 0.92 to 22.40 mg/kg, whereas the amounts of nitrate for all the cheese varieties tested ranged from 0.68 to 22.40 mg/kg [25]. Additionally, Buket et al.'s (2007) investigation on Turkish white cheese revealed nitrate ranges of 0–24.02 mg/kg [21]. Tudor et al.'s 2007 investigation of nitrate in traditional Romanian cheese reported a range of 0.87 – 17.52 mg/kg for nitrate [4]. Greek cheeses' nitrate and nitrite levels were measured by Kyriakidis et al. (1998), and the findings revealed that the nitrate concentration ranged from 0.7 to 13.1 mg/kg [10,14,13]. (Kirovska-Cigulevska 2002) discovered that the nitrate levels in food products from the Republic of Macedonia, including cheeses, ranged from 8.5 to 11.8 mg/kg for sheep's milk cheese and from 12.9 to 21.6 mg/kg for cow's milk cheese [21].

4.4. Conclusion

The 39 samples under study had nitrate averages of 6.07 mg/kg 19.60% of the samples had nitrate concentrations higher than 10 mg/kg. Nitrate concentrations exceeding 10 mg/kg have the potential to revert cheese milk to an unsanitary state, given the lack of adequate control over the production procedures of white cheeses. Even while the portion of nitrate that can be attributed to cheese consumption is tiny relative to the total amount of nitrate that can be consumed through food, we cannot ignore the potential function that these compounds can play as precursors to the production of the carcinogenic N-nitroso compounds. Gloria et al. (1997) reported that the amounts of N-nitroso dimethylamine and N-nitroso diethylamino in the cheese milk positively correlated with the amount of nitrate added to the studied cheeses.

Standard specifications, whether international or Libyan, are similar in determining the level of nitrates in cheeses. Measured nitrates are categorized into three types: added nitrates, residual nitrates, and measured nitrates. Added nitrates refer to those introduced during cheese production. Some countries allow the addition of nitrates at levels not exceeding 20 mg per 100ml, such as Canada and Brazil, while Egypt has a limit of 10 mg per 100 ml. Others, like the United States, prohibit the addition of nitrates during processing [32]. European standards rely on measured nitrates, which should not exceed 150 mg/kg for cheese [31], a regulation that aligns with Egyptian, Gulf, and Libyan specifications [27].

The maximum and minimum levels of various types of cheese studied were measured as follows: for the first type, spread cheese, the lowest concentration was measured at 6.98mg/kg, and the highest concentration was 37.93mg/kg, with an average of 15.11mg/kg. For cream cheese, the lowest concentration was 20.46mg/kg, while the highest concentration was 174.20mg/kg, with an average of 48.32mg/kg. The mozzarella cheese showed the best measured values, with the lowest concentration at 3.49mg/kg and the highest at 40.18mg/kg, with an average 23.17mg/kg. The semi-soft cheese had a lower concentration of 20.59mg/kg and a higher concentration of 50.16mg/kg, with an average of 33.55mg/kg. The last type, salty feta cheese, recorded the highest concentrations of nitrate, with a minimum concentration of 151.24mg/kg and a maximum of 171.20mg/kg, averaging 158.28mg/kg. This type exceeded the permissible limits.

Lastly, in the event that the naturally occurring nitrate in cheese milk cannot be eliminated, stringent controls can be implemented to reduce concentration excesses, with a focus on the requirement for legislation that establishes the highest allowable limits for nitrate, as is the case in many other nations.

4.3.1. Recommendations:

- A cheese can be added to children’s foods after 2 Years.
- The favorite Cheeses is squeezed cheese and spread cheese because they have less nitrate.
- A void eating salt cheeses daily and replace it with less salty cheese.

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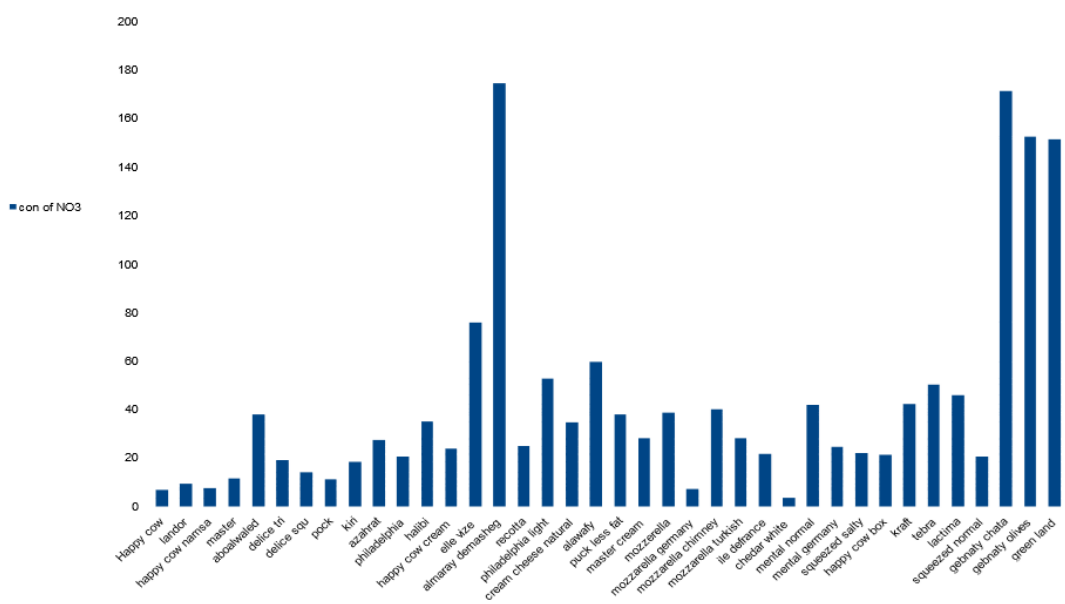
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