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Review Paper

Occurrence of nitrates in milk products-review

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ABSTRACT

This review explores the role of nitrates, primarily sodium nitrate and potassium nitrate, in cheese production, particularly for semi-hard and hard cheeses. Nitrates are added as food additives to control microbial growth and prevent defects caused by gas-producing bacteria. The initial spike of nitrate levels during cheese production decreases over time, with trace levels remaining in the final product. Various regulations around the world dictate nitrate levels in cheese, with notable differences between the EU and the US. Nitrates can also originate from natural and environmental sources, influenced by cows' diets and agricultural practices. Furthermore, nitrates act as antimicrobial agents during cheese ripening, supporting product quality by inhibiting spoilage bacteria. There are several techniques used to analyse nitrates (NO3-), including spectrophotometers, Raman spectrometry, infrared and per fluorometrically-interferon- irradiated (IR and FTIR) spectroscopy, AAS, fluorophotography, chemical luminance, mass spectrometry, MECA, EPR, and NMR, Gas-Liquid Chromatography (GLC) with Electron-Capture Detector (ECD), High-Performance Liquid Chromatography (HPLC) with UV detection.

1. Introduction

Nitrates, primarily in the form of sodium nitrate ($NaNO_3$) or potassium nitrate (KNO_3), are used in cheese production, particularly for certain semi-hard and hard cheeses like Emmental, Gouda, Swiss type and Edam. These compounds are added as food additives to control microbial growth, specifically to prevent the "late-blowing" defect caused by gas-producing bacteria such as *Clostridium tyrobutyricum* and *Clostridium butyricum*. These bacteria can produce carbon dioxide during cheese ripening, leading to undesirable cracks, slits, or holes in the cheese (*Zamrik,* 2013). The addition of nitrate salts causes an initial spike in nitrate levels in the cheese; however, during ripening, the concentration continues to decrease to trace levels. Good manufacturing practices must be applied by law to avoid big natural content in milk. Plant-based ingredients, such as truffles, clover, herbs, peppers, seeds, are naturally high in sodium nitrate, and could increase the amount of nitrates and nitrites, when added to certain cheeses, especially cheese spreads or processed cheese (*Genualdi et al*, 2018). The use of nitrates in cheese production is controversial due to potential health risks, primarily related to the formation of N-nitrosamines, which are genotoxic and carcinogenic compounds. Nitrates can be reduced to nitrites in cheese, and nitrites can react

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with secondary amines (present in proteins or other compounds) to form N-nitrosamines, some of which (e.g., N-nitrosodiethylamine) are classified as carcinogenic by the International Agency for Research on Cancer (IARC) (https://www.foodtimes.eu/food-system-en/nitrites-nitrates-and-nitrosamines-efsa-revises-risk-analysis/), (*Grey et al*, 1979).

2. Nitrates as cheese additives

Nitrates are typically added to cheese milk at concentrations ranging from 10 to 30 g per 100 kg of milk in some regions, though regulations vary. In the European Union, up to 150 mg/kg of nitrates can be added to cheese milk, while the CODEX General Standard for Food Additives sets a maximum residual limit of 50 mg/kg in the final cheese product. In contrast, the United States does not approve nitrates as food additives in cheese production. (*Zamrik*, 2013). Raw cow's milk may contain between 1 and 5 mg/L of nitrate and less than 0.1 mg/L of nitrite (*Indyk and Woolard*, 2010). Nitrates can also be present in cheese from endogenous sources, such as milk contaminated with nitrates from water, forage, or fertilizers, or from plant-based ingredients in processed cheeses. Beet root is natural source of nitrates (*Blom-Zandstra*, 1989) and it is used as livestock feed. Studies have found nitrate levels in cheese ranging from below detection limits to 26 mg/kg, with most samples (93%) containing less than 10 mg/kg, indicating primarily endogenous sources (*Genualdi et al*, 2018). The internal level of nitrites in milk depends strongly on the quality of nutrients provided to the cows (*Topçu et al,* 2009; *Cristea,* 2008). Diets rich in nitrate but poor in fermentable energy reduce the capacity of rumen microbes to fully convert nitrate into ammonia, resulting in nitrite accumulation. These elevated nitrite concentrations can enter the bloodstream and be secreted into milk, where they affect both the technological properties of cheese and the survival of nitrite-tolerant pathogens. Nitrates and nitrites in milk products can originate from cow diet, ingestion of nitrates from feed or water (*Taghawi et al.,* 2025; *Genualdi et al*, 2020; *Branova et al*, 1993). Environmental contamination originates from fertilizers, agricultural runoff, or industrial waste increasing nitrate in water supplies

3. Nitrate's mechanism of action

Nitrates act as antimicrobial agents by being reduced to nitrites by nitrate-reducing bacteria during cheese ripening. Nitrites then inhibit the growth of spoilage bacteria, ensuring product quality (*Topçu et al*, 2006). Ingested nitrate (NO_3^-) is eliminated through the kidneys in around 60 % of cases, whereas notable 25 % undergoes active concentration within the salivary glands. Saliva that contains nitrate (NO_3^-) is released into the oral cavity, where facultative anaerobic bacteria use nitrate (NO_3^-) reductase enzymes to transform nitrate (NO_3^-) into nitrite (NO_2^-) (*Tiso and Schechter*, 2015). Following ingestion, nitrite-rich saliva is further metabolized non-enzymatically in the stomach's acid to produce nitric oxide (NO) followed by numerous biological effects of NO. Nitrates prevent spoilage in cheese in two main ways: Inhibition of microbial growth and control of gas-producing bacteria. Inhibition of microbial growth occurs when nitrates (NO_3^-) are reduced to nitrites (NO_2^-) by enzymes (e.g., nitrate reductase) produced by certain bacteria naturally present in cheese or starter cultures. Nitrites are the active antimicrobial agents. Nitrites disrupt the metabolism of spoilage-causing and pathogenic bacteria by interfering with their respiratory processes, enzyme activity, or cell membrane integrity. This creates an unfavorable environment for microbial growth. Nitrites may also react to form nitric oxide (NO), which further enhances antimicrobial effects by damaging bacterial DNA or proteins. Nitrates are particularly effective at preventing late blowing, a spoilage defect in cheese caused by gas production (e.g., carbon dioxide or hydrogen). Late blowing results in undesirable cracks, fissures, or off-flavors in cheese. By inhibiting gas-forming bacteria, as mentioned earlier *Clostridium spp*, also *Streptococcus bovis*, nitrates help maintain the structural integrity and sensory quality of cheese during ripening. Nitrates work in conjunction with other cheese preservation factors, such as low water activity, high salt content, low pH (due to lactic acid production by starter cultures), and competitive exclusion by beneficial bacteria. This creates a hurdle effect, making it difficult for spoilage organisms to thrive.

Microorganisms targeted by nitrates belong to Clostridium species, coliforms and other pathogenic bacteria. *Clostridium tyrobutyricum* anaerobic, spore-forming bacterium is a primary cause of late blowing in cheese. It ferments lactic acid into butyric acid, carbon dioxide, and hydrogen, leading to off-flavors (rancid or fermented smells) and textural defects. Nitrates effectively inhibit its growth, preventing these issues. Other *Clostridium spp*. *C. sporogenes* or *C. butyricum* can also contribute to spoilage, and nitrates suppress their activity. Coliform bacteria, such as *Escherichia coli* or Enterobacter species, are associated with early gas production and off-flavors in cheese, often due to poor hygiene during milk collection or cheese production. Nitrites derived from nitrates inhibit their proliferation. Other pathogenic bacteria particularly *Listeria monocytogenes* can survive in cheese, mostly in soft or semi-soft varieties. Nitrites have been shown to reduce its growth, enhancing cheese safety. *Staphylococcus aureus* are successfully limited by nitrates can and this pathogen is a concern in cheeses made from raw milk or under improper hygienic conditions. Nitrates may also have some effect against *Salmonella spp*. and certain strains of *Bacillus spp*., although their primary role is against anaerobes like Clostridium. Scientific studies and reviews, such as those published in journals like International Dairy Journal and Journal of *Masoud et al,* 2011 demonstrated that nitrates effectively controlled *Clostridium tyrobutyricum* in Gouda cheese, reducing gas formation during ripening. A review of *Van Horde et al*, 2010 highlighted the role of nitrates in preventing late blowing and their synergistic effect with starter cultures in semi-hard cheeses. Research also indicates that the efficacy of nitrates depends on factors like cheese pH, salt content, and ripening conditions. For example, nitrates are more effective in cheeses with a pH below 6.0, where nitrite formation is enhanced. The use of nitrates in cheese is regulated due to concerns about nitrosamine formation (potentially carcinogenic compounds). For example, the European Union permits nitrate use in specific cheeses (e.g., up to 150 mg/kg in Gouda or Edam), but levels are tightly controlled. Some cheese producers use lysozyme (an enzyme) or specific starter cultures to control Clostridium and other spoilage organisms as alternatives or complements to nitrates.

Nitrates prevent cheese spoilage by being converted to nitrites, which inhibit the growth of spoilage and some pathogenic microorganisms, particularly *Clostridium tyrobutyricum* (responsible for late blowing), but coliforms, and pathogens like *Listeria monocytogenes* and *Staphylococcus aureus* can survive in presence of nitrites. Their antimicrobial action disrupts bacterial metabolism and prevents gas production, preserving cheese quality. Nitrite-tolerant pathogens (*L.monocytogenes, E. coli, Salmonella spp.*) are food safety concern, regardless to nitrites because nitrite in cheese does not guarantee inhibition. Scientific studies confirm their efficacy, especially in hard and semi-hard cheeses, when used within regulatory limits.

4. Benefits of nitrates

Nitrates (NO_3^-) enhance cardiovascular health by lowering blood pressure. Nitric Oxide (NO) relaxes and widens arteries and veins, resulting in enhanced blood circulation and tissue oxygen supply throughout the body (*Bentley et al.*, 2017). They are also used in the form of dietary supplements, particularly in the context of sports nutrition. Over the past few years, the role of nitrates (NO_3^-) in promoting human physical condition and preventing diseases has received increasing attention from researchers and health professionals (*Affourtit et al.,* 2015). Dietary nitrate (NO_3^-) influences its effects by producing nitric oxide (NO) in the physiological system. Numerous physiological and pathological processes, such as cardiac contraction, immunomodulation, prevention of platelet aggregation, vasodilation, and neuronal transmission, depend on the intra and intercellular signaling molecule nitric oxide (NO) (*Kanno et al*, 2004).

5. Main risks associated with nitrates in cheese production

The use of nitrates in cheese production is controversial due to potential health risks, primarily related to the formation of N-nitrosamines, which are genotoxic and carcinogenic compounds. Nitrates can be reduced to nitrites in cheese, and nitrites can react with secondary amines (present in proteins or other compounds) to form N-nitrosamines, some of which (e.g., N-nitrosodiethylamine) are classified as carcinogenic by the International Agency for Research on Cancer (IARC) (https://www.foodtimes.eu/food-system-en/nitrites-nitrates-and-nitrosamines-efsa-revises-risk-analysis/), (*Grey et al,* 1979). Studies indicate that while N-nitrosamine formation is well-documented in cured meats due to high protein content and cooking at high temperatures, there is less data on their presence in cheese. Potential of nitrosamines exists, particularly in cheeses with added nitrates, as the protein-rich environment can facilitate nitrosamine formation under certain conditions (e.g., specific pH or microbial activity (*Grey et al,* 1979). Cheeses often contain lower levels of nitrosatable precursors and may have protective compounds (e.g., antioxidants like vitamin C), which reduce nitrosamine formation. Modern cheese-making practices, such as improved milk quality and pasteurization, have reduced the need for nitrate addition, further lowering this risk

(Johnson, 2017). Cured meats are nevertheless high in nitrite (NO_2^-) and nitrate (NO_3^-) contents and could be the reason for developing colo-rectal cancer (*Shakil et al,* 2022). Because of risk posing to nitrosamines from nitrates, The European Union (EU), and the World Health Organization's (WHO) Joint FAO/Food and Drug Administration (FDA) Expert committee on Food additives (JECFA), have established an ADI (Acceptable Daily Intake) of nitrates (NO_3^-) for a person with body weight (body-weight-kg-1) of 0–3.7 parts per million (b.w.), equivalent to 222 parts per million (ppm) Nitrate (NO_3^-) daily for an adult 60 kg and ADI of 0–0.07 mg kg-1 b.w. for nitrites (NO_2^-) (WHO/JECFA, 2015). While cheese contributes only a small fraction of dietary nitrate intake (0.2% of ADI in some studies), high consumption of nitrate-rich foods (e.g., vegetables, processed meats, and cheese) could lead to exceeding the ADI, particularly in vulnerable populations like infants. (*Karwowska M. and Kononiuk A.,* 2020; *Roila et al,* 2018). A study in Belgium found that cheese and meat products contributed 0.2% and 6% of the nitrate ADI, respectively, with vegetables being the primary source (50%). In Syrian white cheese, nitrate levels ranged up to 26 mg/kg, but nitrite levels were generally below detection limits, suggesting minimal risk from cheese alone (*Zamrik,* 2013). However, cumulative exposure from multiple sources remains a concern, especially if nitrate levels in cheese are not strictly controlled. (*Roila et al.,* 2018). Methemoglobinaemia is a condition where nitrate is reduced to nitrite in the body, oxidizing hemoglobin to methemoglobin, which impairs oxygen transport. This is particularly concerning for infants, who are more susceptible due to their immature enzymatic systems. Acute toxicity occurs at nitrate levels of 2–9 g (2000–9000 mg), which is unlikely from cheese consumption alone but could be a risk from contaminated water or other sources. (*Zamrik*, 2013) Studies suggest that the low nitrate levels in cheese (typically <10 mg/kg) do not pose a significant risk for methaemoglobinaemia. A U.S. study found no nitrite concentrations above 0.1 mg/kg in 64 cheese samples, indicating minimal risk from nitrites in cheese (*Genualdi et al,* 2018). Nitrate levels in raw milk are generally low, typically ranging from less than 1 to 12 mg/kg. Nitrite is often practically absent or at trace levels (*Zbikowski et al,* 2000). An old study found that nitrate content in milk from cows given drinking water with 0–180 mg/L nitrate remained very low, suggesting minimal impact from water contamination (*Kamemerer et al,* 1992). Another study reported a rapid increase in nitrate content (up to 5.6 mg/L in one cow and 3.2 mg/L in another) after administering sodium nitrate, but levels returned to a normal range of ~1 mg/L within 24 hours (*Geissler et al,* 1991). After administering potassium nitrate (KNO_3) to dairy cows, residual nitrate in milk reached 34.6 mg/L two hours after a 150 g dose, with elevated levels persisting up to 38 hours (*Baranová et al,* 1993). In a study from Shijiazhuang, China, liquid milk had an average nitrate content of 10.05 mg/kg (ion chromatography) and 9.95 mg/kg (cadmium column reduction method), with about 14% of samples exceeding regulatory limits. (*Ping et al.,* 2013). In Taiwan, nitrate concentrations in milk ranged from 0.3 to 417.7 mg/kg, with an average of 92.7 mg/kg. Nitrite was below the detection limit 0.07mg/kg in all samples. (*Yeh et al.,* 2013). In the United States, milk powders showed average nitrate concentrations of 17 ± 12 mg/kg, with nitrite detected above 2 mg/kg in 5 out of 39 brands, including both dairy and plant-based (soy and coconut) powders (*Genualdi et al,* 2020). In Taiwan, milk powder had an average nitrate content of 25.74 mg/kg (ion chromatography) and 25.66 mg/kg (cadmium column reduction method) (*Ping et al,* 2013). In a survey of commercial dry milk samples, nitrate levels were slightly higher (1–3 ppm more nitrate-nitrogen) in products from direct-fired (gas) dryers compared to indirect-heated (steam) systems (*Manning et al,* 1968). In Iran, infant formulas contained nitrate levels ranging from 0.221 to 1.347 mg/kg (mean 0.645 mg/kg) and nitrite from 0.045 to 0.263 mg/kg (mean 0.151 mg/kg). Baby foods had nitrate levels from 0.24 to 1.93 mg/kg (mean 0.99 mg/kg) and nitrite from 0.04 to 1.45 mg/kg (mean 0.36 mg/kg). All were below WHO acceptable daily intake limits (*Taghawi et al.,* 2025). Human breast milk nitrate levels vary by production stage: Colostrum (days 1–3 postpartum): 0.19 mg/100 mL nitrate, 0.08 mg/100 mL nitrite. Transition milk (days 3–7): 0.52 mg/100 mL nitrate, 0.001 mg/100 mL nitrite. Mature milk (days >7): 0.31 mg/100 mL nitrate, 0.001 mg/100 mL nitrite (*Hord et al,* 2011).

Nitrates in cheese can originate from contaminated water, forage, or fertilizers used in dairy farming, reflecting poor hygienic conditions or environmental pollution. Adding nitrates to mask poor hygiene during cheese production is a concern, as it may compensate for inadequate sanitation practices (*Zamrik,* 2013; *Topçu et al,* 2006). Research on Turkish and Syrian cheeses noted that nitrate levels could be elevated due to external contamination (e.g., nitrate fertilizers or water). For example, Turkish white cheese samples showed nitrate levels up to 17.19 mg/kg, attributed to both added nitrates and environmental sources. Stricter controls on milk quality and production hygiene are recommended to minimize reliance on nitrate additives (*Topçu et al,* 2006). *Koréneková et al.* (2000), studied nitrate dynamics in Emmental cheese production, finding that nitrate levels decreased significantly from 81.2 mg/kg in milk to 3.3 mg/kg in the final product, with most nitrates passing into whey. This suggests that residual nitrates in cheese are minimal under controlled conditions. *Genualdi et al.* (2018) developed a method to measure nitrates and nitrites in U.S. cheeses, finding low levels up to 26 mg/kg for nitrates, none for nitrites above 0.1 mg/kg, indicating that endogenous nitrates dominate and intentional addition is rare in the *U.S. EFSA* (2022) updated risk analysis on N-nitrosamines in food, confirming their genotoxic and carcinogenic potential and emphasizing the need to monitor nitrate use in cheese and other products. *Zamrik* (2013) noted that nitrate use in Syrian white cheese (10–30 g/100 kg milk) could pose health risks if doses exceed recommended levels, particularly due to potential carcinogenicity. *Topçu et al.* (2022) found nitrate levels in Turkish cheeses ranging from 0.68–17.19 mg/kg, with higher levels in sheep's milk cheeses, highlighting the need for stricter controls to avoid masking poor hygiene. The risk of N-nitrosamine formation in cheese is lower than in cured meats due to lower protein content and the presence of protective antioxidants, but it remains a concern requiring further research (*Grey et al,* 1979). Regulatory limits and improved hygiene practices have reduced nitrate use, but environmental contamination and cumulative dietary exposure remain challenges. (*Zamrik,* 2013; *Topçu et al.,* 2022).

6. Methods for nitrates detection

There are several techniques used to analyze nitrates (NO_3^-), including spectrophotometers, Raman spectrometry, infrared and per fluorometrically-interferon- irradiated (IR and FTIR) spectroscopy, AAS, fluorophotography, chemical luminance, mass spectrometry, MECA, EPR, and NMR. Every method has its fundamental tenets, detection range, detection limits, sample throughput percentage, benefits, and drawbacks (*Lin et al,* 2019). Methods for determining nitrates in cheese typically involve dissolving the cheese in water, followed by reducing nitrate to nitrite and quantifying the nitrite content using a spectrophotometer. The International Dairy Federation standard method is a common approach. Several methods exist for detecting nitrate in cheese. These include spectrophotometry, ion chromatography, and cadmium reduction followed by spectrophotometry (*Tudor et al.* 2007). Ion chromatography separates the ions in a sample (including nitrate and nitrite) based on their affinity for a stationary phase. The separated ions are then detected, providing a means to quantify their concentrations (*Genualdi et al,* 2018). Cadmium Reduction involves reducing nitrate to nitrite using a cadmium column resulting nitrite is then measured using spectrophotometry by ISO14673-1;2004. Gas-Liquid Chromatography (GLC) with Electron-Capture Detector (ECD) involves nitration of a compound, extraction, and analysis by GLC-ECD (Tanaka et al, 1982). High-Performance Liquid Chromatography (HPLC) with UV detection uses HPLC to separate compounds and UV detection for nitrate, while UV/vis spectrophotometry is used for nitrite (*Srivichien et al.,* 2015). The choice of method depends on the specific needs and capabilities of the laboratory. Some methods, like cadmium reduction, may involve the use of toxic substances. Other methods, like ion chromatography, can provide more accurate and sensitive results for detecting low levels of nitrates and nitrites.

7. Conclusion

Good manufacturing practices must be applied by law to avoid big natural content in milk. Plant-based ingredients, such as truffles, clover, herbs, peppers, seeds, are naturally high in sodium nitrate, and could increase the amount of nitrates and nitrites, when added to certain cheeses, especially cheese spreads or processed cheese. Risk of nitrates exposure from cheese and milk products is rather low, comparing to cured meat and other protein rich products. Methods for detection of nitrates are various and they should be adjusted to needs of the laboratory sample throughput as well as to expected content of nitrates in samples.

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