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# Naturally occurring nitrates in cheese

Scientific excellence
Industry applicability
Strategic networking
Global influence


## Summary

Nitrates can be present in cheese naturally. The addition of nitrates during the cheesemaking process is not common, as milk quality and processing technologies have addressed the original purpose of such additions, i.e. to control the outgrowth of certain spoilage bacteria that can lead to cheese quality problems. When used in certain cheese varieties, the natural degradation during aging leads to low nitrate and nitrite content, whilst still ensuring good quality of cheese of good quality. The following factsheet provides an overview of the topic and how modern cheesemaking practices have sought to avoid the need for nitrate/nitrite addition.


## Background

Raw cow's milk may contain between 1 and 5 mg/L of nitrate and less than 0.1 mg/L of nitrite (1). The intrinsic levels depend on the quality of feed materials of the cows (2, 3). In the past, unintentional nitrate contamination had been reported because of residual cleaning and sanitizing chemicals used on dairy processing equipment (1, 4). Good manufacturing practices must be applied by law to avoid this. Plant-based ingredients, such as truffles, 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 (5).

## Role of nitrates and nitrites during cheese making

Since the 1830's, sodium nitrate or potassium nitrate have been used in the manufacturing of certain cheeses, sometimes in combination with potassium nitrite or sodium nitrite (6). Nitrate is often added into the curd or whey mixture, after most of the whey has been removed, and this acts as a precursor of nitrite. The addition causes an initial spike in nitrate levels in the cheese; however, during ripening, the concentration continues to decrease to trace levels. Furthermore, the nitrite concentration always remains low (<1 mg/kg) in the finished cheese (7, 8, 5). The compounds prevent germination of the spores of *Clostridium tyrobutyricum*, in sweet-curd type cheeses.

*Clostridium butyricum* and *Cl. tyrobutyricum* often originate from silage used for animal feed. They can be present in concentrations of 105 spores of bacteria per gram of feed, and the spores may be transferred to the milk. Cheeses, which have higher initial pH values (>5.8), slower rate of salt absorption (2-3 days of salting), and are stored at higher (>7°C) temperature (9, 10, 11) are susceptible to growth of *Cl. butyricum* and *Cl. tyrobutyricum*.

Although the inhibitory mechanism of nitrates and nitrites on Clostridium growth and germination has not been fully established, it seems to rely on a range of reactive intermediates such as NO, N2O3, peroxynitrite (ONOO-), nitrogen dioxide (NO2), and RS-NO (Figure 1). Such compounds act on several target molecules and structures via N-nitrosylation, S-nitrosylation, disulphide formation, and lipid peroxidation, thus compromising the cellular functions (12).

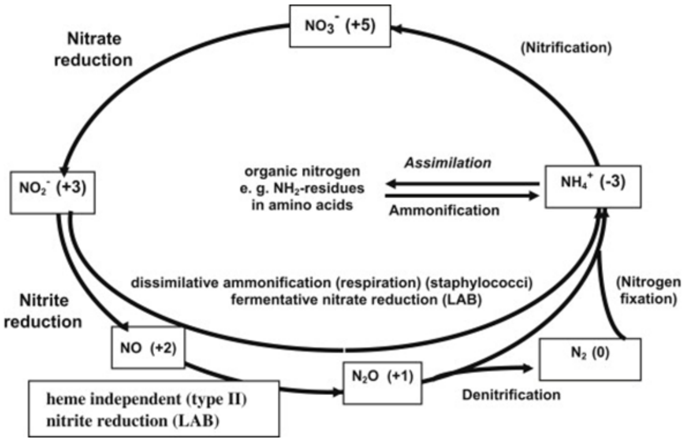
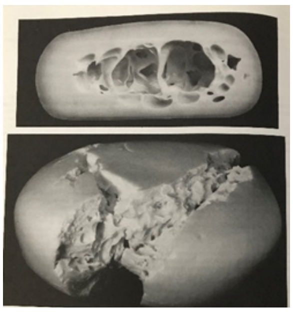



Figure 1. The Nitrogen cycle (12)

## Potential quality defects in absence of nitrates and nitrites



A quality defect leading to cheese spoilage, late blowing, is caused by *Cl. tyrobutyricum* spores in cheese through butyric fermentation i.e. fermentation of lactate to butyrate, CO2, and H2. The pressure of accumulated gases causes cracks and splits (Figure 1), which are generally accompanied by an unpleasant aroma and rancid flavor. The bacterium is not harmful to humans and animals. Cheeses made from milk with the spores of other silage-associated *Clostridium species*, e.g., *Cl. beijerinckii, Cl. sporogenes* and *Paenibacillus species*, do not produce late-blowing.

Figure 2. Example of “blowing” in sweet-curd type cheeses (9)

## Health concerns with nitrates and nitrites

The practice of using nitrates in cheese-milk is sometimes questioned, because the residual nitrate in food is reduced to nitrite from bacteria in the saliva, and under certain conditions, e.g. the acid gastric conditions or heat treatment, reacts with secondary amines to form nitrosamines (13). Nitrosamines originate from secondary biogenic amines in foods, which can be formed into products high in protein or free amino acids because of the bacterial decarboxylation.

Formation of N-nitroso compounds is accelerated by the presence of nitrosatable compounds and inhibited by vitamin C and other antioxidants. Nitrosamines have been classified as probably carcinogenic to humans (13). Therefore, Acceptable Daily Intake of nitrate and nitrite has been determined to be, respectively, 0 to 3.7 mg nitrate and 0.06 mg nitrite per kg body weight per day (14).

While CODEX General Standard for Food Additives (15) considers the use of sodium and/or potassium nitrates in manufacturing of different varieties of cheese safe, it has limited the maximum permitted level of residual nitrate to 35 ppm in cheese. Regulations in different countries may vary (Table 1).

Table 1. Maximum allowable nitrate/nitrite in some countries

| **Country** | **Nitrate/Nitrite (maximum)** | **Reference** |
|---|---|---|
| Brazil | 20 g of nitrate per 100 L of milk | (16) |
| Canada | Permitted in limited varieties; 200 mg/kg of the milk and milk products used to make the cheese; residual nitrate in cheese not to exceed 50 mg/kg. | (17) |
| USA | Not approved for use as additives in cheese | (18) |
| EU | 150 mg/kg milk;, only hard, semi-hard and semi-soft ripened cheese, calculated as NaNO (sodium nitrate) per kg cheese milk (Note: In the cheese milk or equivalent level if added after removal of whey and addition of water) It is not permitted for use in the manufacturing of organic cheeses) | (19) |

Measurement of nitrates and its degradation compounds are critical for regulatory compliance. Over the years, progress has been made in analytical methodologies, in terms of improved extraction, sensitivity and detection limits of nitrates in cheese (5, 7, 20, 21, 22). The degradation compounds of nitrates, however, are very unstable and quickly reduce into other compounds. It is also impossible to discriminate if the compounds were present naturally or result of addition during cheese making (5). The study of Genualdi et al. (5) found naturally present nitrates and nitrites in cheese at levels of <10 mg/kg and <0.1 mg/kg, respectively, without any addition of the preservative.

## Approaches to minimize nitrates/nitrites in cheese

While the use of sodium nitrates can be helpful in manufacturing certain types of cheeses, cheesemakers in many countries have been able to discontinue the use of nitrate compounds, or reduce usage level because of hygiene improvements on the dairy farm, thereby reducing the number of spores in the raw milk. Avoidance of the presence of *Cl. tyrobutyricum* is of utmost importance and it can be achieved using milk from cows that are not fed with silage, or fed with good quality silage, maintaining sanitary conditions in the cowshed, and good hygienic conditions during milking. The standard milk pasteurization treatment does not eliminate spores, but bactofugation with high g-force (10,000 x g) or microfiltration can reduce spore numbers, avoiding the need for nitrate compounds. *Bactofugation* removes 98.7% of the anaerobic spores originally present in milk. Double *bactofugation* can increase the reduction level to 99% or more (23).

Bacteriocins (including nisin), are sometimes employed to prevent *Cl. tyrobutyricum* outgrowth and hence, for example, has been approved in some countries, such as the United States, for use in cheese spreads to inhibit the outgrowth of *Cl. tyrobutyricum* or *Cl. botulinum spores* (24). Lysozyme is often used to prevent growth of *Cl. tyrobutyricum* spores (6). Furthermore, if the cheese variety allows for the use of higher salt content (>5% salt in moisture phase), and ripening for shorter time at lower temperature (<7°C), this could be used as other approaches to control butyric fermentation (6).

## Nitrate and nitrite in cheese and the human diet

Vegetables, especially leafy vegetables, are the dominant source of dietary nitrate in humans, and contribute 60-80% of the total nitrate intake. Other sources of nitrate are drinking water (15-20%) and other foods, including animal-based products (10-15%) to which nitrate and nitrite are added as preservatives and to enhance flavour and colour (26). Leafy vegetables are naturally high in nitrates; thus, the Commission Regulation (EC) No. 1881/2006 (19) has established much higher limits of nitrate levels (Table 2) when compared to dairy products. Interestingly, the largest source of nitrite in humans (about 93 %) is endogenous to human body, i.e. its presence in human saliva (27).

| Foodstuff | Range of Nitrate (mg/kg) |
|---|---|
| Fresh spinach | 3500 |
| Fresh lettuce | 3000 – 5000 (depending on growing conditions) |
| Rucola (Rocket) | 6000 – 7000 (depending on harvesting time) |
| Processed cereal based foods | 200 |

Table 2: Maximum level of nitrate contamination in plant-based foodstuff (19).

## Conclusion

Nitrates are generally not used in the cheesemaking process, however when they are used, the levels are low and they are quickly broken down into other compounds (including nitric oxide, dinitric oxide and/or nitrogen gas). The milk quality from the farm has been consistently improving, which itself is an important factor upon preventing the quality defect of late blowing. The amount of residual nitrates and nitrites in cheeses is not a major contributor of nitrates and nitrites found in the human diet and will not lead to exceeding the Acceptable Daily Intake (0 to 3.7 mg nitrate and 0 to 0.06 mg nitrite per kg body weight per day) (14). However, the dairy sector must continue the rigorous efforts to ensure that the highest standards in farming (e.g. silage quality and milking hygiene) and manufacturing practices in the cheese-making facilities are met, in order to guarantee the best quality cheese to the consumers.

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