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Research on evolution of nitrite and nitrate content regarding milk processing in scalded cheese

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Abstract:

Nitrates have an insignificant toxicity. They are considered virtual toxic substance because they convert into nitrogen, which have a toxic potential well known. Milk contaminated with nitrates must be severely restricted.
Objectives of our research took into account the wholesomeness of milk to determine nitrates and nitrites. Sixty samples of raw milk (15 samples per season) were taken into consideration, to see the level of nitrites and nitrates from processed milk for consumption. To study the remanence of nitrates and nitrites in the finished product 60 samples of cheese were also analyzed.
Following the conducted research, no evidence has exceeded the maximum permissible limit for nitrates and nitrites which is max. 10 mg/ liter milk. For samples of cheese were not found values above 10 ppm that is the maximum permissible limit for nitrates and nitrites in cheese. Nitrates are concentrated in cheese 1.3 times. The content of nitrites in the cheese falls by 1.9 times, from 0.03367 mg/ l in milk to 0.01728 mg/ kg in cheese.

Keywords: residues of nitrites and nitrates, Griess method, colorimetric method with m-xylenol, cheese

1. Introduction:

Nitrates and nitrites added to food can cause formation of cancerous N-nitroso compounds, whereas exposure to perchlorate is especially emphasised as an important risk factor for newborns' health. (SUNGUR [17]) Some epidemiological studies linking intake of nitrate and nitrite with gastric cancer in humans indicated a positive correlation. (SIMION & al. [16]).

The main sources of contamination of milk are: water, placing fraudulent ammonium nitrate in milk and nitric acid used to remove "Stone Milk" on pasteurizers with plate. Nitrites from milk are highly toxic, especially for children. Nitrites concentrations grow 5-10 times in severe heat treatment, therefore, to feed infants and children there have been developed separation techniques through semi-permeable membrane. (ŢIBULCĂ [21]).

Water polluted with nitrates and nitrites can get into milk especially during cleaning operations of equipment and containers. The main source of water contamination is the ammonium nitrate used as basic chemical fertilizer in agricultural practice (SINDILAR [18], JIMBOREAN & al. [11]).

Nitrates and nitrites get in animals through feed and water. Nitrogen is absorbed by plants in the form of either ammonium (NH^{4+}) or nitrate (NO^{3-}), and its accumulation is influenced by a series of factors that are depending on the species, cultivar, age and soil conditions (SIMION & al. [16]). Although animals can ingests large amounts of nitrates and nitrites, organic tissues contain relatively small amounts, due to rapid metabolism and excretion in the urine. Additionally, in ruminant a large part of nitrates and nitrites is subject to changes by ruminal microflora. (BONDOC [2], COSTIN [7]). The nitrate may naturally present in milk and the level of it depends on the quality of feeding materials (i.e. water, feeds) of cows. (TOPÇU [19]).

Nitrogen content in milk is very low. According to Walker, milk content in nitrogen is between the values of 20 and 40 mg nitrate/ liter. This variation in the analytical data is determined by nature of the feed consumed. (WALKER [23]).

In preparations of milk in 2006, the Brasov area, the average content of nitrite was 0.009 g/ kg and ranged from not detected to 0.64 mg/ kg, and the average nitrate content was 0.95 mg/ kg and ranged from not detected and 12.95 mg/ kg. (CRISTEA [4]).

In some countries may be added nitrate in the milk used to prepare cheese to prevent bloating caused by coliform, but mainly to prevent latter bloating produced by some clostridia. Part of the nitrate is removed in the whey and some are converted intoharmless compounds in the maturation process. (COSTIN [6], JIMBOREAN [10]) In addition, the residual quantity decreases gradually during maturation (GUŞ [8], BONDOC [2]).

In Brazil, the use of these additives is permitted at maximum levels of 50 mg/kg. The basis of the previously validated method is the quantitative reduction of nitrate to nitrite through cadmium column and spectrophotometric determination after nitrite diazotation with sulphanilic acid/alpha-naphtol reagent. From all samples analyzed, 38 (88.37%) showed neither a nitrate nor nitrite detectable content; 5 samples (11.63%) presented nitrate, 4 of them being above the tolerated level and only one (2.33%) showed detectable nitrite, however below the permissible limit. The majority of samples did not show nitrate or nitrite at detectable levels. However, though probably safe from a toxicological point of view, the results can implicate an increased risk of bacteriological contamination. (SERAPHIM [15]). In other researches, the highest level of nitrate and nitrite was found in green cheese (17.52 mg/kg and respectively at 21.16 mg/kg) obtained from sheep milk. (TUDOR & al. [20]).

Objectives of our research are to assess the level of nitrites and nitrates from processed milk for their consumption and remains detectable in the final product: cheese.

2. Materials and Methods:

Cheese preparation:

Cheeses have the primary feature of the technological process to obtain curd which, after maturation and slicing, is subjected to scalding temperature of 72-74°C (BANU [1] CODOBAN [5], COSTIN [6], ŢIBULCĂ [22], JIMBOREAN [10]).

Curd preparation involves the following: milk reception, cleaning and standardization of milk, milk pasteurization and cooling, preparation for coagulation which consists of adding calcium chloride (clotting ability of milk increases, it adds 10 to 20 g/ 100 liters of milk) and selected cultures of lactic bacteria (*Lactococcus lactis* ssp. *lactis*, *Streptococcus thermophilus* and *Lactobacillus casei*). *Lactococcus lactis* ssp. *lactis* is a regular agent of milk spontaneous acidification, hydrolyzing lactose into lactic acid. *Streptococcus thermophilus* produce a weak acidification and a specific flavor. *Lactobacillus casei* is a proteolytic bacteria and plays a role in cheese maturation. (BANU [1], CODOBAN [5], COSTIN [6], ŢIBULCĂ [22], JIMBOREAN [10]). Pasteurization is able to destroy essential microflora, enzymes and pathogens in milk. It should be noted that inactivation level of microorganisms depends on the amount of microorganisms, growth phase and other factors. (CIPROVICA [3]).

The coagulation of milk takes place at 32 ... 35°C, for 30 - 40 minutes. Processing the curd is then made, and the resulting curd maturates at 18-26°C for 6 ... 10 hours after a pH

4.8 to 5.0 is achieved in curd. Mature curd is cut into slices having a thickness of 0.3 to 0.5 cm and a width of 3-6 cm and is scalded at 72 ... 74°C for 50-60 s in brine with 10 - 12% salt. The slurry is allowed to air in molds, and is maturated. Storage is done at 4 ... 6°C and φ = 85-90%. (GUZUN [9]).

Experimental design:

The reference method for the determination of nitrite in milk is Griess method. The reference method for nitrates is colorimetric method with meta-xylenol. This method determines total nitrogen. Nitrate content is calculated by the difference between total nitrogen and the nitrite determined by Griess method and expressed in equivalent nitrate. (JIMBOREAN & al. [12], MUREŞAN [14]).

Determination of nitrate and nitrite is done through the following methods: determination of nitrate with diphenylamine (nitrates form with diphenylamine a blue colored complex; color intensity is proportional to the concentration of nitrates in the analyzed sample and is measured colorimetrically); the method for determining the indole nitrite (nitrite formed with indole in sulfuric acid medium, a pink-colored complex; the intensity of the color is proportional to the nitrite concentration in the sample to be analyzed and is measured colorimetrically). In case of dispute the method used is to reduce nitrate to nitrite in cadmium copper environment. The reagents used must be of quality for analysis or of equivalent quality. Water must be distilled or of equivalent purity, free of nitrates and nitrites. (ŢIBULCĂ [21]).

3. Results and discussions:

NO_3^- and NO_2^- limits for milk and measures to be taken are:

For NO_3^- :

- lack NO_3^-, but correlated with NO_2^- it is freely admitted in consumption;
- up to 10 mg/ liter is allowed in consumption without restrictions;
- from 10 mg/ liter to 100 mg/ liter is excluded from food for children under one year and is admitted for adults;
- more than 100 mg/ liter is excluded from consumption and is processed in fermented cheese products.

For NO_2^-:

- lack NO_2^- but correlated with NO_3^- is freely allowed freely in consumption;
- less than 10 mg/ liter is allowed without restrictions in consumption;
- more than 10 mg/ liter, regardless of the amount of NO_3^- is excluded from consumption and is processed in fermented chesses. (BONDOC [2])

The determined levels of nitrates and nitrites in raw milk are shown in Table 1.

Table 1. Variation of nitrates and nitrites content in raw milk

Crt. No	Season	Samples/ season	Nitrates NO_3^-, mg/1			Nitrites NO_2^-, mg/l		
			0-0,05	0,051-0,1	> 0,1	0-0,05	0,051-0,1	> 0,1
1.	A	15	3	12	-	10	5	-
2.	W	15	-	14	1	8	7	-
3.	Sp	15	4	11	-	13	2	-
4.	Su	15	1	14	-	13	2	-
TOTAL		60	8	51	1	44	16	-
%		100	13,3	85	1,7	73,3	26,7	-

legend: A/ autumn; W/ winter; Sp/ spring; Su/ summer.

Of the 60 milk samples investigated no evidence has exceeded the maximum permissible limit for nitrates and nitrites which is max. 10 mg/ l milk. Nitrates had an average (±) of 0.0623 ± 0.004678 mg/ l and a coefficient of variation of 58.16%. The determined values ranged between 0.001 and 0.3 mg/ l. Statistically analyzing the nitrates variation, per seasons, shows the following:

- In autumn, nitrates, had an average (±) of 0.05287 ± 0.006306 mg/ l and a coefficient of variation of 46.19%. The determined values ranged between 0.001 and 0.092 mg/ l.
- In winter, nitrates, had an average (±) of 0.08867 ± 0.01547 mg/ l and a coefficient of variation of 67.57%. The determined values ranged between 0.057 and 0.3 mg/ l.
- In spring, nitrates, had an average (±) of 0.0552 ± 0.004762 mg/ l and a coefficient of variation of 33.41%. The determined values ranged between 0.001 and 0.092 mg/ l.
- In summer, nitrates, had an average (±) of 0.0007488 ± 0.05247 mg/ l and a coefficient of variation of 5.53%. The determined values ranged from 0.05 to 0.061 mg/ l.

Nitrites had an average (±) of 0.03367 ± 0.003034 mg/ l and a coefficient of variation of 69.81%. The determined values ranged between 0.0 and 0.08 mg/ l. Statistically analyzing the nitrites variation, per seasons, shows the following:

- In autumn, nitrites, had an average (±) 0.036 ± 0.007024 mg/ l and a coefficient of variation of 75.56%. The determined values ranged between 0.01 and 0.08 mg/ l.
- In winter, nitrites, had an average (±) of 0.05 ± 0.004781 mg/ l and a coefficient of variation of 37.03%. The determined values ranged between 0.02 and 0.08 mg/ l.
- In spring, nitrites, had an average (±) of 0.02667 ± 0.00504 mg/ l and a coefficient of variation of 73.19%. The determined values ranged between 0.01 and 0.07 mg/ l.
- In summer, nitrites, had an average (±) 0.022 ± 0.004995 mg/ l and a coefficient of variation of 87.94%. The determined values ranged between 0.0 and 0.06 mg/ l.

In Romania it is forbidden the use of nitrite in milk and milk products. Nitrites and nitrates are not toxic in their normal concentrations in food. Exceeding certain limits, however, have serious repercussions on the human body (COSTIN [7]).

A large study whose results were presented by Şindilar (ŞINDILAR [18]) included a total of 210 milk samples, of which 95 samples of collected milk, where nitrates had limits of variation between 0 and 7.5 mg NO_3^-/ l, with an average of 2.7 NO_3^-/ l.

The limits of variation of nitrate and nitrite in studied cheese samples are shown in Tables 2 and 3.

Table 2. Variation limits of *nitrates* content in pressed cheese samples investigated

Crt. No	Season	Samples	Nitrates, ppm							
			0-0,1	%	0,11-1	%	1,1-10	%	> 10	%
1.	A	15	14	93,3	1	6,7	-	-	-	-
2.	W	15	9	60	6	40	-	-	-	-
3.	Sp	15	14	93,3	1	6,7	-	-	-	-
4.	Su	15	15	100	-	-	-	-	-	-
Total		60	52	86,7	8	13,3	-	-	-	-

legend: A/ autumn; W/ winter; Sp /spring; Su/ summer

As shown in table 2 values of nitrate were recorded as follows:

- 93.3% of cheese samples had values of nitrate between 0-0.1 ppm;
- 6.7% of cheese samples had values of nitrate between 0.11 to 1 ppm.

Table 3. Variation limits of *nitrites* content in pressed cheese samples investigated

Crt. No	Season	Samples	Nitrites ppm							
			0-0,01	%	0,011-0,1	%	0,11-10	%	> 10	%
1.	A	15	8	53,3	7	46,7	-	-	-	-
2.	W	15	4	26,7	11	73,3	-	-	-	-
3.	Sp	15	8	53,3	7	46,7	-	-	-	-
4.	Su	15	8	53,3	7	46,7	-	-	-	-
Total		60	28	46,7	32	53,3	-	-	-	-

legend: A/ autumn; W/ winter; Sp /spring; Su/ summer

As shown in table 3 of nitrite values were recorded as follows:

- 46.7% of cheese samples had values of nitrite ranging from 0 to 0.01 ppm;
- 53.3% of cheese samples had values of nitrite ranging from 0.011 to 0.1 ppm.

No evidence had values > 10 ppm that is LMA for nitrates in cheese, according to Reg. EC 1881/2006.

Statistically, nitrates had an average (±) of 0.08 ± 0.005797 ppm and a coefficient of variation of 56.13%. The determined values ranged between 0.0012 and 0.36 ppm.

Statistically analyzing the nitrates variation, per seasons, shows the following:

- Infall, nitrates, had an average (±) of ± 0.004898 0.06721 ppm and a coefficient of variation of 28.22%. The determined values ranged between 0.0012 and 0.078 ppm.
- In winter, nitrates, had an average (±) of ± 0.008054 0.06873 ppm and a coefficient of variation of 45.39%. The determined values ranged between 0.0014 and 0.11 ppm.
- In spring, nitrates, had an average (±) of 0.1139 ± 0.01823 ppm and a coefficient of variation of 62.01%. The determined values ranged between 0.068 and 0.36 ppm.
- In summer, nitrates, had an average (±) of ± 0.005988 0.07021 ppm and a coefficient of variation of 33.03%. The determined values ranged between 0.0012 and 0.11 ppm.

Statistically, nitrite had an average (±) of ± 0.002279 0.01728 ppm and a coefficient of variation of 102.16%. The determined values ranged between 0.0 and 0,066 ppm.

Statistically analyzing the nitrite variation, per seasons, shows the following:

- In fall, nitrites, had an average (±) of ± 0.009867 0.003567 ppm. The determined values ranged between 0.0 and 0.04 ppm.
- In winter, nitrites, had an average (±) of 0.01867 ± 0.005257. The determined values ranged between 0.0 and 0,066 ppm.
- In spring, nitrites, had an average (±) of ± 0.004916 0.0268 ppm and a coefficient of variation of 71.04%. The determined values ranged between 0.0 and 0.05 ppm.
- In summer, nitrites, had an average (±) of ± 0.003416 0.0138 ppm and a coefficient of variation of 95.87%. The determined values ranged between 0.0 and 0.04 ppm.

No evidence had values > 10 ppm that is LMA for nitrates and nitrites in cheese.

KORÉNEKOVÁ, (2010), in one of his studies on Emmental cheeses, obtained the mean $NaNO_2$ content in untreated and in pasteurized milk of 0.2 and 0.1 $mg \cdot kg^{-1}$, respectively and the mean $NaNO_3$ content of 0.9 and 0.9 $mg \cdot kg^{-1}$ respectively. He also mentioned that the milk with nitrate added had the mean content of 81.2 $mg \cdot kg^{-1}$ $NaNO_3$. The final product had a markedly decreased content of nitrates (3.3 $mg \cdot kg^{-1}$ $NaNO_3$) and nitrites (0.2 $mg \cdot kg^{-1}$ $NaNO_2$) when compared with the values in cheese during maturation (11.3 $mg \cdot kg^{-1}$ $NaNO_3$; 0.4 $mg \cdot kg^{-1}$ $NaNO_2$). The results of his study showed that a considerable quantity of nitrates passed into the whey and that nitrates were added to the milk to prevent the blowing defect of hard cheese by micro organisms. (KORÉNEKOVÁ [13]).

4. Conclusions:

From the 60 investigated milk samples no evidence has exceeded the maximum permissible limit for nitrates and nitrites which is max. 10 mg/ l milk. Nitrates had an average (±) of 0.0623 ± 0.004678 mg/ l. The determined values ranged between 0.001 and 0.3 mg/ l. Nitrites had an average (±) of 0.03367 ± 0.003034 mg/ l. The determined values ranged between 0.0 and 0.08 mg/ l.

Following statistical analysis of the values obtained regarding nitrate for raw milk samples and cheese final product we found statistically significant differences *** ($p < 0.001$) for fall and winter samples and statistically significant * ($0.01 < p < 0.05$) for samples of spring and summer. Overall, registered statistical difference was significant * ($0.01 < p < 0.05$). During processing nitrate concentrate into cheese 1.3 times (from 0.0623 mg/ l in milk to 0.08 mg/ kg in cheese).

Following statistical analysis of the values obtained regarding nitrite for raw milk samples and cheese final product we found statistically significant differences *** ($p < 0.001$) for samples taken in winter distinctively statistically significant ** ($0.001 < p < 0.01$) for the summer samples and statistically significant * ($0.01 < p < 0.05$) for samples of autumn and spring. Overall, registered statistical difference was very significant *** ($p < 0.0001$).
During processing of nitrite, content in cheese decreases by 1.9 times, from 0.03367 mg/ l in milk from 0.01728 mg/ kg in cheese.

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