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THE DETERMINATION OF NITRATE AND NITRITE LEVELS IN TURKISH WHITE CHEESE SAMPLES CONSUMED IN ANKARA REGION

ANKARA BÖLGESİNDE TÜKETİME SUNULAN TÜRK BEYAZ PEYNİRLERİNDE NİTRAT VE NİTRİT DÜZEYLERİNİN SAPTANMASI

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ABSTRACT: In this study, total number of seventy samples were investigated in case of nitrate and nitrate levels in Turkish white cheese which were collected from Ankara local markets and open markets, Turkey. Determination of nitrate and nitrite levels in the cheese samples has been made by spectrophotometric method. The cadmium column which reduced nitrate to nitrite was used for determination of nitrate values in samples. As a consequence, nitrate mean levels found in all cheese samples were within the Turkish Food Codex (TFC). On the other hand nitrite was determined in thirty per cent of white cheese samples.

Keywords : Nitrate, nitrite, cadmium column, white cheese

ÖZET: Bu çalışmada, Ankara bölgesindeki süpermarketlerden ve pazarlardan sağlanan toplam 70 adet Türk beyaz peynirinde nitrat ve nitrit düzeyleri araştırılmıştır. Peynir örneklerdeki nitrat ve nitrit düzeylerinin saptanmasında spektrofotometrik yöntem kullanılmıştır. Örneklerdeki nitrat değerlerinin saptanması için nitratı nitrite indirgeyen kadmiyum kolon kullanılmıştır. Sonuç olarak, bütün peynir örneklerindeki nitrat düzeylerinin Türk Gıda Kodeksinde (TGK) belirtilen değerleri aşmadığı bulunmuştur ve peynir örneklerinin %30'unda nitrit saptanmıştır.

Anahtar kelimeler: Nitrat, nitrit, kadmiyum kolon, beyaz peynir

INTRODUCTION

Nitrate and nitrite occur widely in human and animal foodstuffs, both as intentional additives and as undesirable contaminants (1). Nitrite and nitrate after being metabolized or reduced to nitrite can react with secondary and tertiary amines to form N- Nitroso compounds (2, 3 ,4). N- Nitroso compounds have mutagenic, teratogenic and carsinogenic activity (5, 6, 7).Moreover, excessive intake of nitrite and nitrate in the diet may cause toxic effects since methaemoglobinaemia is produced by oxidation of haemoglobin by nitrite (7).

By bacterial reduction, nitrates may be converted to nitrites during food processing and storage and in saliva and the gastrointestinal tract (8,9,10). The toxicity of nitrate to humans is mainly attributable to its reduction to nitrite. With respect to chronic effects, FAO/WHO Expert Committee on Food Addditives (JECFA) recently re-evaluated the health effects of nitrate/nitrite confirming the previous Acceptable Daily Intake (ADI) of 0-3.7 mg/kg of body weight per day for nitrate ion and establishing an ADI of 0-0.06 mg/kg of body weight per day for nitrite ion. However, it was noted that these ADIs do not apply to infants below the age of 3 months (11). The daily intake of nitrate and nitrite are 50-120 mg, 2-5 mg, respectively in human. 10% of these are taken from milk and milk products (12). Gloria et al. (13) has reported nitrate levels in the cheese increases with the

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amount of nitrate added to cheese milk. Positive correlation was observed between the level of nitrate added to cheseemilk and the levels of N-nitrosodimethylamine and N-nitrosodiethylamine in the cheese in the same study.

According to the Turkish Food Codex, the maximum contaminant level of nitrate could not be more than 10 mg/kg alone in cheese (14).

In our research we used Turkish white cheese. This is a popular cheese in Turkey; it represents 60-80% of total cheese production. It is a brined (or a pickled) cheese variety with a soft or semi-hard texture and a salty, acid taste. Several characteristics of Turkish white cheese are compared to other white cheese varieties such as Feta and Domiati. Based on its moisture content (55-65 g/100 g), Turkish white cheese has a soft variety (15). There is no definition of Turkish white cheese based on texture in the Turkish Standards Institute (16).

Our aim was to determine the nitrate and nitrite levels in five different firm's cheese samples, cheese samples of unknown firm sold in Ankara local markets and cheese samples sold in open markets and to evaluate whether nitrate levels were within the Turkish Food Codex values or not.

MATERIALS AND METHODS

Samples Collection

In this study, total of seventy samples have been analysed, of which 50 samples from A, B, C, D, E firms, 10 samples of unknown firm (F) from Ankara local markets and 10 samples from open markets (G). All samples were kept at +4°C.

Method of Analysis

All reagents were of analytical grade. The extraction and determination procedure for analysis of cheese were based on the method described by AOAC (17). Determination of nitrite and nitrate values have been made by spectrophotometric method. The cadmium column which reduced nitrate to nitrite was used for determination of nitrates in cheese samples (17,18). The spectrophotometric method is most oftenly used for determination of nitrites. The same method can be used for determination of nitrates, after their reduction to nitrites. The detection limit was ≥ 1 ppm NO_3 for this method.

Analysis of Nitrate and Nitrite

Briefly, a 15 g cheese sample was first extracted with 35 ml hot water at 50°C. After precipitation of proteins with 5 ml $ZnSO_4$ and 6 ml 2% NaOH, the NO_2 were determined directly and it was also determined after the reduction of nitrate to nitrites by cadmium column (glass tube, 300 x10 mm id; cadmium 80-100 mesh and length, 80-100 mm) in the filtrate. For the colouring elicited in both the reduced eluated and unreduced filtrate 10 ml sulphanilamide and HCl of 1-naphthylamine was added and after that waited for 25 minutes. The absorbance is measured at a wavelength of 525 nm by Beckman DU650 Spectrophotometer. Standards of nitrite were measured in the same way. Nitrite calibration curve was determined, using series of concentrations of nitrite as 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml, 20 µg/ml, 30 µg/ml. This standard curve had an r-value of 0.9992.

The NO_3 ion is calculated by the difference between the concentration of NO_2 in the unreduced filtrate and the reduced eluated through the cadmium column. The results are expressed in mg/kg of $NaNO_3$ and $NaNO_2$.

Statistics

Student's t-test and One-Way Anova test were conducted for statistical comparisons (19).

RESULTS AND DISCUSSION

For mean recovery, 2 mg/kg, 6 mg/kg, 10 mg/kg nitrite was added to cheese samples and afterwards nitrite levels were measured in these samples. The mean recovery was calculated as 99.28 % for nitrite.

Spectrophotometric method was found to be reproducible, as similar results were obtained when the same operator used the same equipment. All analysis were repeated twice for each sample. The concerning nitrate and nitrite levels are shown in Table 1, 2. Morever, the comparison between the values of nitrate in white cheese samples (mg/kg) and the limit value of regulation are shown in Table 3.

Table 1. The levels of nitrate in white cheese samples (mg/kg)

	N	$\bar{X}$ ± S E	Min	Max
A	10	8.7920 ± 1.2011	2.51	14.23
B	10	4.4340 ± 0.9747	0	11.60
C	10	4.7290 ± 1.2411	0	12.23
D	10	7.1260 ± 2.2261	0	24.02
E	10	6.0380 ± 1.5424	2.62	18.55
F	10	4.3590 ± 2.3385	0	21.84
G	10	8.9160 ± 2.0408	0	18.70
Total	70	6.3420 ±0.6619	0	24.02

Table 2. The levels of nitrite in white cheese samples (mg/kg)

	N	$\bar{X}$ ± S E	Min	Max
A	10	0.1890 ± 0.0616	0	0.57
B	10	0.3180 ± 0.1791	0	1.84
C	10	0.2560 ± 0.1849	0	1.90
D	10	0.3090 ± 0.1869	0	1.96
E	10	0.6160 ± 0.1357	0	1.25
F	10	0.5860 ± 0.2810	0	2.68
G	10	1.0580 ± 0.3290	0	2.88
Total	70	0.4760 ± 0.08328	0	2.88

Table 3. The comparison between the values of nitrate in white cheese samples (mg/kg) and the limit value of regulation

	N	$\bar{X}$ ± S E	t
A	10	8.7920 ± 1.2011	1.006-
B	10	4.4340 ± 0.9747	5.710***
C	10	4.7290 ± 1.2411	4.247**
D	10	7.1260 ± 2.2261	1.291-
E	10	6.0380 ± 1.5424	2.569*
F	10	4.3590 ± 2.3385	2.412*
G	10	8.9160 ± 2.0408	0.531-

- : p>0.05 (insignificant)

* : p<0.05 (The difference between the mean nitrate level determined in white cheese samples of E firm and unknown firm (F) and the TFC values ;10 mg/kg)

** : p<0.01 (The difference between the mean nitrate level determined in white cheese samples of C firm and the TFC values ;10 mg/kg)

*** : p<0.001 (The difference between the mean nitrate level determined in white cheese samples of B firm and the TFC values ;10 mg/kg)

Table 1 shows that the mean values of nitrate were found as 8.7920 ± 1.2011 mg/kg, 4.4340 ± 0.9747 mg/kg, 4.7290 ± 1.2411 mg/kg, 7.1260 ± 2.2261 mg/kg, 6.0380 ± 1.5424 mg/kg, 4.3590 ± 2.3385 mg/kg and 8.9160 ± 2.0408 mg/kg, respectively in white cheese samples of A, B, C, D, E firms, unknown firm (F) from local

markets and open markets (G). The maximum and minimum levels were determined as 0 mg/kg and 24.02 mg/kg. In addition to this, the values of mean nitrite were found as 0.1890 ± 0.0616 mg/kg, 0.3180 ± 0.1791 mg/kg, 0.2560 ± 0.1849 mg/kg, 0.3090 ± 0.1869, 0.6160 ± 0.1357 mg/kg, 0.5860 ± 0.2810 mg/kg and 1.0580 ± 0.3290 mg/kg, respectively, in white cheese samples of A, B, C, D, E firms, unknown firm (F) from local markets and open markets (G). The maximum and minimum levels were determined as 0 mg/kg and 2.88 mg/kg (Table 2). Results of the analysis were evaluated through the guidelines of Turkish Food Codex (14). According to this, for cheese, the maximum contaminant level of nitrate could not be more than 10 mg/kg alone. Table 3 shows that the difference between the nitrate values in white cheeses of E firm, unknown firm (F) and the TFC values were significant ($p<0.05$). In addition to this, the nitrate values in white cheeses of C firm and B firm were also significant as $p<0.01$ and $p<0.001$, respectively.

Our data revealed that nitrate mean levels found in all cheese samples were within the Turkish Food Codex values. Nitrite was determined in 30% white cheese samples. Since there is not a standard method for white cheese production it usually is produced in dairy farms in non-hygienic conditions and these results indicate that such conditions could result in contamination.

Several authors have reported the detection of nitrate and nitrite in Turkish cheese. Among these, there are some data reporting lower levels than those reported in the present study. For instance, Özçetin (20) has investigated and has determined the nitrate levels as 0.01-1.47 mg/kg in a total of 35 white cheese examined. However, in this investigation, nitrite levels are higher than in our study. Some researchers reported that even higher nitrate and nitrite levels were detected compared to the results of this study. For instance, İrkin (21) determined 19% nitrate and nitrite which is high contamination rate in the white cheese samples. Türkdoğan et al. (22) reported nitrate and nitrite mean levels as 26.06 mg/kg and 4.14 mg/kg, respectively in the 50 herb-enriched cheese samples in Van region. Morever, Aygün (23) has investigated and has determined the mean nitrite levels as 1.8 mg/kg in a total of 52 carra cheese examined. However, nitrate levels of this study are lower than in our study.

The levels of nitrate and nitrite in cheese samples has been investigated in several countries. Balcerska et al. (24) reported nitrate and nitrite levels as 5.2-70.7 mg/kg and 0.4-2.6 mg/kg, respectively from a total of 24 cheese samples. Lopez-Y-Lopez Leyton et al. (25) reported nitrate mean level as 9.12 ± 2.9 mg/kg. Morever, Kyriakidis et al. (26) reported mean nitrate and nitrite levels as 0.7-13.1 mg/kg and 0-1.7 mg/kg, respectively from a total of 140 cheese samples.

Regional differences and storage conditions can also affect nitrate and nitrite levels. Also, the unconscious use of nitrate and nitrite during the production can also affect the nitrate and nitrite residue levels. Thus, the nitrat and nitrite levels that were determined in white cheese in our study could be the result from contamination or the unconscious usage of nitrate and nitrite during production. Nitrates and nitrites are considered harmful to health, because of the possibility of formation of carcinogenic nitrosamines from nitrite. For this reason, their levels must be controlled in food industry.

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