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RESEARCH ARTICLE

# Nitrite Content of Wheat Samples from Different Regions of Turkey

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***Nitrite Content of Wheat Samples from Different Regions of Turkey***

***Summary***

*Wheat is a staple food in many countries, including Turkey, which is one of the major wheat-producing countries in the world. The average daily consumption of wheat and wheat products by the Turkish population is about twice that of Western countries. The main source of exogenous human exposure to nitrite is food, including vegetables, drinking water, cured meat, and cereal products. Several studies indicate an association between dietary intake of nitrite and the occurrence of many cancers. Although there are several studies on the nitrite levels of the different foodstuffs, the studies based on the nitrite content of cereal and cereal products are limited. The present study aimed to compare the nitrite content of wheat samples grown and consumed in different regions of Turkey. Forty samples of wheat were collected from the Turkish Grain Board Office, and nitrite concentrations were determined by a spectrophotometric method. The mean nitrite levels of wheat (mean ± SEM) were found to be 6.37 ± 0.38 mg/kg for the East Anatolia region (n=10), 8.31 ± 1.38 mg/kg for the Central Anatolia region (n=23), and 7.14 ± 3.54 mg/kg for the West Anatolia regions (Marmara/Aegean) (n=7). The differences between the groups were not statistically significant (p>0.05). Estimated daily intake levels are below the given acceptable daily intake (ADI) by the Joint Expert Committee on Food Additives (JECFA). However, overall consumption of nitrite by the organism in one day with other foods must also be considered. It can be concluded that the nitrite levels in different cereals, vegetables and fruits should be monitored routinely to prevent food-borne hazards and to protect susceptible populations such as infants, young children and the elderly.*

***Key Words:*** *Wheat, nitrite, food toxicity, Turkey.*

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***Türkiye'nin Farklı Bölgelerinden Alınan Buğday Örneklerinin Nitrit İçeriği***

***Özet***

*Buğday Türkiye dahil birçok ülkede üretilen başlıca besindir ve Türkiye, dünyada buğday üreten başlıca ülkelerden biridir. Türk popülasyonunun buğday ve buğday ürünlerini ortalama günlük tüketme oranı Batı ülkelerinin yaklaşık iki katıdır. İnsanların ekzojen olarak nitrite ana maruziyeti sebzeler, içme suyu, işlem görmüş etler ve tahıl ürünleri ile olmaktadır. Çok sayıda çalışma günlük nitrit alımı ve birçok kanser türünün ortaya çıkması arasındaki ilişkiye işaret etmektedir. Farklı gıdaların nitrit düzeylerine ilişkin bir çok çalışma bulunmakla birlikte tahıl ürünlerinin nitrit içeriğini inceleyen çalışmalar sınırlıdır. Bu çalışma, Türkiye'nin değişik bölgelerinde üretilen ve tüketilen buğday örneklerindeki nitrit düzeylerini karşılaştırmak amacıyla yapılmıştır. 40 buğday örneği Toprak Mahsulleri Ofisi'nden toplanmış ve buğday örneklerindeki nitrit düzeyleri spektrofotometrik bir yöntemle belirlenmiştir. Ortalama nitrit düzeyleri (ortalama ± SEM) Doğu Anadolu Bölgesi (n=10) için 6.37 ± 0.38 mg/kg, İç Anadolu Bölgesi (n=23) için 8.31 ± 1.38 mg/kg ve Ege/Marmara Bölgeleri (n=7) için 7.14 ± 3.54 mg/kg olarak belirlenmiştir. Gruplar arası farklar istatistiksel olarak anlamlı bulunmamıştır (p>0.05). Tahmin edilen günlük alım düzeyleri Gıda Katkı Maddeleri Uzman Komitesi (JECFA) tarafından verilen ADI değerlerinin altındadır. Bununla birlikte, diğer gıdalar ile birlikte bir gün içinde organizmanın tüm nitrit tüketimi dikkate alınmalıdır. Sonuç olarak, gıdalardan gelebilecek tehlikelerin önlenmesi ve bebek, çocuklar ve yaşlılar gibi hassas popülasyonların güvenliğinin sağlanması için tarımsal ürünlerin nitrit içerikleri rutin olarak izlenmelidir.*

***Anahtar kelimeler:*** *Buğday, nitrit, gıda toksisitesi, Türkiye*

## INTRODUCTION

Nitrites and nitrates possess a unique position in human toxicology (1). They are both ubiquitous in the environment and can be formed from nitrogenous compounds by microorganisms present in the soil, water, saliva, and the gastrointestinal tract. Nitrates occur naturally in soil containing nitrogen-fixing bacteria, decaying plants, septic system effluent, and animal manure. Other sources of nitrate include nitrogenous fertilizers and airborne nitrogen

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compounds emitted by industry and automobiles (2). Nitrate penetrates into soil and remains in groundwater for decades (3,4).

Nitrites and nitrates are important antimicrobial agents against botulism, as well as food preservatives and flavoring/coloring agents in meat and fish products. These compounds, on the other hand, can cause adverse cellular effects, although the extent and significance of health risk resulting from exposure to nitrites and nitrates remain unclear1. The toxicity due to nitrate ingestion is relatively low. On the other hand, 5% to 20% of nitrates taken from different sources including water and several foodstuffs are endogenously converted to nitrites, which have higher toxicity and are thought to be responsible for several adverse health effects (5-7).

One of the most important effects of nitrites is on hemoglobin. Hemoglobin's function in oxygen carriage is so overwhelmingly important that its other functions in physiology have been obscured. The transport of oxygen requires ferrous hemoglobin to bind. Many oxidizing agents including nitrites oxidize ferrous hemoglobin to ferric hemoglobin, which is unable to bind oxygen. The resulting compound is methemoglobin and the condition is referred to as methemoglobinemia, which presents clinically with symptoms and signs of tissue hypoxia (8,9). Nitrites also have a vasodilatory effect that can further contribute to the problem of nitrite-induced anoxia (10). Infants, younger than 5-6 months of age, pregnant women, nursing mothers, and the elderly are more susceptible to nitrite-induced methemoglobinemia (11). Patients with glucose-6-phosphate dehydrogenase deficiency, a common condition in some Asian and Mediterranean populations, may present with methemoglobinemia and intravascular hemolysis following exposure to oxidants such as nitrites (12,13).

Nitrosamines are ubiquitous in our environment and diet. Nitrosamines are formed from the reaction of nitrite with primary, secondary, or tertiary amines in an acid medium, and many of them are known to cause cancer (14-16). A high dietary intake of nitrite and nitrate has been implicated as a risk factor for human cancer, and formation of nitrosamines in the stomach is correlated with nitrate levels in food and water (17,18). There is thought to be an association between gastric cancer and nitrosamines. Diet can be the major determinant of risk(19). Many nitroso-compounds are carcinogenic in animals and most probably in man. The International Agency for Research on Cancer (IARC) has classified four N-nitroso compounds (NOCs) as probably (Group 2A), and another NOCs as possibly (Group 2B) carcinogenic in humans(20). As there is a genotoxic mechanism of action, no safe level has been determined for NOCs. Moreover, some NOCs induce oxidative stress and lipid peroxidation and are thought to cause gastric, esophageal and hepatocellular carcinomas (21).

A number of biochemical changes, functional impairments and histopathological lesions have been observed in nitrite-treated rats. An orally administered 25–100 mg of sodium nitrite kg-1 diet to Balb/c mice for 21 days resulted in a dose-dependent decrease in lymphocyte percentages, concanavalin A- and lipopolysaccharide-induced lymphocyte proliferation, natural killer cell activity against WEHI-164 target cells, as well as IgM and IgG titers against injected sheep erythrocytes, and the immunosuppressive effect of sodium nitrite is reversible after cessation of exposure (22). While nitrates are not as toxic as nitrites, ingestion of large amount of nitrates may cause severe gastroenteritis. Methemoglobinemia, anemia and nephritis caused by prolonged exposure to small amounts of nitrates likely result from its reduction to nitrite (23).

Concerning the toxic effects of nitrite exposure and the high consumption of wheat and wheat-based products in the Turkish population, the present study was aimed to compare the nitrite content of wheat samples grown and consumed in different regions of Turkey and to evaluate the toxicological outcomes.

## MATERIALS and METHODS

### Sample Collection

Forty samples of wheat harvested in the summer of 2002-2003 were obtained from the Turkish Grain Board Office, the largest wheat collection, distribution and storage organization in Turkey. The wheat samples were crumbled by a Teflon blender and wheat flours were put in nylon bags with shackles to prevent contamination with air, and nitrate/nitrite contamination from the dampness of air was thus deterred. All samples were stored at –20°C until the analysis.

**Chemicals**

All chemicals used in this study were analytical grade (Sigma Co., St. Louis, MO, USA and Merck Co., Darmstadt, Germany). Ultra-high pure distilled water was used in the analytical work.

**Standards**

Stock nitrite solution was prepared by dissolving 50 mg sodium nitrite in 100 ml of ammonium hydrochloride buffer (prepared from HCl and ammonia; pH=9.7). A working solution containing 50 µg/ ml sodium nitrite was prepared daily by proper volume of stock solution. Standard solutions containing 0.025, 0.05, 1, 1.5, 2, 5, and 10 µg/ml sodium nitrite were obtained with dilution.

**Extraction Procedure**

For each sample, 10 g of ground wheat was used for the nitrite analysis. Hot water (40 ml) was added on the sample and blended for 5 min in a blender. The mixture was heated to 75°C for the prevention of ascorbic acid interference. The solution was transferred to a volumetric glass and 50 ml hot water and 12 ml sodium hydroxide (2% w/v in water) was added and blended again for another 10 min. Zinc hydroxide (10 ml) (7.2% w/v in water) was added and the mixture was shaken for 5 min. The next step was to add 5 ml sodium hydroxide and the mixture was blended for another 5 min. Distilled water (83 ml) was added and mixed for 5 min. The last volume was 200 ml. The mixture was filtered using filter paper (Whatman No. 1) until the filtrate was completely clear.

**Determination of Nitrite Levels**

The Griess method was employed with slight modifications. The principle of the method was based on the reaction between nitrites and sulfanilic acid (1% w/v in 30% acetic acid) and Marshall's reagent to produce a purple-colored compound(24). The absorbance was measured at 550 nm. In order to detect the nitrite level in the samples, 1 ml of the clear diluted filtrate was used. Ammonium hydrochloride buffer (0.9 ml) and 0.5 ml of 60% acetic acid were added on the filtrate tubes. Sulfanilic acid (0.5 ml) and Marshall's reagent (0.5 ml) (N-(1-naphthyl) ethylenediamine hydrochloride; 0.1% w/v in 60% acetic acid) were added, then diluted to 5 ml with water, and the mixture was vortexed for 30 sec. The mixture was incubated in the dark for 25 min. The same procedure was applied to sodium nitrite standard solutions, and the absorbance was determined at 550 nm using a spectrophotometer (Shimadzu 160 UV, Japan).

The nitrite levels in the samples were calculated using the calibration curve (Fig. 1). The detection limit of the method was 0.025 µg/ml. Recovery studies were performed on blank samples of wheat spiked with of 0.5 and 1 µg/ml nitrite. The average recovery was found to be 95.33 ± 0.8%. Within-day precision was 3.8% coefficient of variance and between-day precision was 5.59%. The nitrite content of the sample was presented as mg of nitrite per kg of the sample.

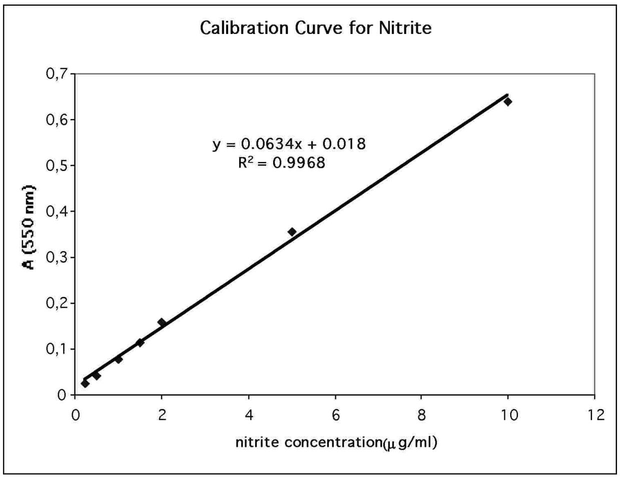


**Figure 1 :** Calibration curve of nitrite standards.

**Statistical Analysis**

All results were expressed as mean ± standard error of mean (SEM). The differences among the groups were evaluated with Kruskal-Wallis analysis of variance. P-values <0.05 were considered statistically significant.

**RESULTS**

Forty samples of wheat collected from different regions in Turkey were analyzed for nitrite levels by the spectrophotometric method. Distribution of wheat samples collected according to the regions is given in Fig. 2. The mean concentration of nitrite in all of the samples was determined to be 7.76±1.04 mg/kg. Fifty-five percent of the samples were found to contain nitrite under the detection limit of method. The mean nitrite levels of wheat were calculated to be 6.37±0.38 for the East Anatolia region (n=10), 8.31±1.38 for the Central Anatolia region (n=23), and 7.14±3.54 mg/kg for the West Anatolia regions

(Marmara/Aegean) (n=7) (Fig. 3). The differences between the groups were not found to be statistically significant, possibly due to the high standard deviation. The minimum and maximum levels along with median levels of nitrite in the samples are also shown in Table 1.

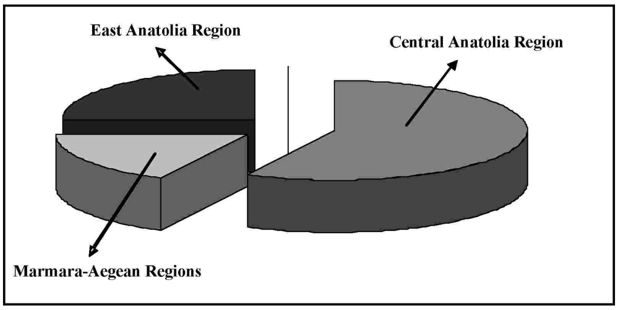


**Figure 2 :** Distribution of wheat samples collected according to the regions.

**Table 1.** Nitrite levels of wheat samples from different regions of Turkey

| Region | Number of Sample | | Nitrite Level (mg/kg) | |
|---|---|---|---|---|
| | Non-detectable | Detectable | Mean ± SEM | Median |
| Central Anatolia | 11 | 12 | 8.31 ± 1.38 | 8.00 |
| Marmara-Aegean | 4 | 3 | 7.14 ± 3.54 | 5.97 |
| East Anatolia | 7 | 3 | 6.37 ± 0.38 | 5.90 |
| **Overall** | 22 | 1 | 7.76 ± 1.04 | 6.85 |

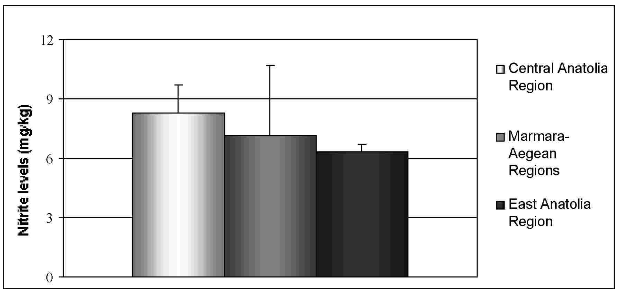


**Figure 3 :** Nitrite levels in the wheat samples from different regions of Turkey. Values are given as mean ± SEM.

## DISCUSSION

Interest in the measurement of nitrates and nitrites in certain agricultural products has increased in recent years. Due to the intense use of synthetic nitrogen fertilizers and livestock manure in modern agriculture, food (particularly vegetables) and drinking water may contain higher levels of nitrates and nitrites than in the past (25). Although vegetables are seldom a source of acute toxicity, they account for more than 70% of the nitrates in a typical human diet (26). Green leaf vegetables (e.g. watercress, celery, collard greens, lettuce, broccoli, parsley, fennel, and spinach) are thought to contain the highest nitrite levels. In addition, cauliflower, radish, potatoes, and carrots may contain high amounts of nitrite. Nitrite content of fruits is low. Strawberries seem to have the highest content of nitrite while apples and pears seem to have the lowest (5). The remainder of the nitrate in a typical diet comes from drinking water (about 21%) and from meat and meat products (about 6%), in which sodium nitrate is used as a preservative and color-enhancing agent. For infants who are bottle-fed, however, the major source of nitrate exposure is the drinking water used to dilute formula (27).

The level of nitrate and nitrite in plants varies according to the kind of the plant and nitrogen content of the soil(28). Furthermore, seasonal differences as well as temperature and humidity changes in the harvesting of the products may cause changes in the nitrate/nitrite content, and nitrate/nitrite levels of agricultural products may change from year to year according to the nitrate/nitrite content of the soil in which the plant grows (5).

Although there are several studies on the nitrite content of vegetables, fruits and cured-meat products, the studies based on the nitrite content of cereal and cereal products are limited (5,26,29,34). Similar results were obtained in a survey of the nitrite content of fruit, vegetables and cereals grown in Slovenia during 1996-2002. The concentration of nitrite in cereals was found to be 8.9 mg/kg in that study. There are also only a limited number of studies on nitrite levels of different foodstuffs in Turkey(30-36). Furthermore, monitoring studies on nitrite and nitrate contents in cereal products as well as other foods have not been carried out routinely by the Ministry of Agriculture in Turkey. We have reported the nitrite content of baby foods and infant formulae and more recently the nitrite content of meat-based and chicken-based bouillons (30,37). Turkdogan et al. (32) determined that herb-enriched cheese contained 4.14 µg/g nitrite, while bread baked by wood fire contained 0.82 µg/g nitrite and bread baked by animal manure contained 3.02 µg/g nitrite in Van, Turkey. They suggested that a nitrite- and nitrate-rich diet could significantly affect the development of endemic upper gastrointestinal cancers in that city. It is a fact that diet can be the major determinant of risk in several diseases, including cancer. Dogan et al. (36) measured nitrite levels ranging between 0.0-0.261

mg/kg in different fruits grown in the Van district and reported that nitrite and nitrate concentrations of fruits delivered to street markets from the other regions were higher than those of the fruits grown in their own region.

Turkey is one of the major wheat-producing countries in the world. The average daily consumption of wheat and wheat products by an average Turkish person is about two times as high as in most Western countries. Daily consumption of these products is estimated to be approximately 400 g, corresponding to almost 50% of the daily diet (38). The major wheat-producing areas are in the Central Anatolia region. The wheat samples grown in this region are generally distributed to the other regions. Therefore, ~60% of the samples analyzed were collected from these regions.

The mean nitrite level in all of the wheat samples was determined to be 7.76±1.04 mg/kg. The differences between the groups were not statistically significant. The high nitrite content of the wheat samples may be attributed to several reasons. The first and most important may be the high amounts of nitrogenous fertilizer and animal manure used in agriculture throughout Turkey. High temperature and humidity in the storage of wheat samples after harvesting may be another reason. Contamination of samples with several nitrogen-containing compounds may be another important cause of these high levels. The high nitrite content of wheat from the West Anatolia region in particular indicates the important effect of industrialization on the total nitrite content of the soil and therefore the plant.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Scientific Committee on Food (SCF) have proposed an acceptable daily intake (ADI) for nitrite of 0-0.07 mg/kg (body weight, b.w.) per day, while the Environmental Protection Agency (EPA) has set a reference dose (RfD) of 0.10 mg nitrite nitrogen per kg b.w. per day (equivalent to 0.33 mg nitrite ion/kg b.w. per day)(39). With a simple calculation, if a 70 kg person consumes 400 g of wheat and wheat products everyday, he may intake approximately 3.104 mg of nitrite, which is below the given ADI by the JECFA. However, overall consumption of nitrite by the organism in one day with other cereals must also be considered. Water, vegetables and other cured-meat products may have high nitrite levels. Chronic intakes of nitrite in excess of the ADI may lead to increased risk of mild to moderate methemoglobinemia, especially for susceptible populations such as young children and the elderly. Furthermore, high nitrite intake favors the formation of NOCs in the gastrointestinal tract.

## CONCLUSION

In view of the above, strict law enforcement in our country should be undertaken regarding the conservation of soil and nutrient management issues with respect to the contamination of waterways and wetlands by nitrogenous compounds, since the exposure of soil to high nitrogenous fertilizers and to industrial wastes/compounds increases day by day. Furthermore, farmers should be warned and educated by regulatory authorities regarding good agricultural practices to decrease the toxic compound content of the plants. New soil management guidelines that respond to pollution of the environment should be established, since there are growing concerns regarding food safety and possible detrimental effects on human health.

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