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The Measurement of Nitrite and Nitrate Contents of Tomato from the Vast Plain of Kermanshah State of Iran

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ABSTRACT: The presence of nitrite and nitrate in food chains is associated with numerous health problems, including stomach, gullet and gallbladder cancer and methaemoglobinaemia. Because of the adverse effects of nitrite and nitrate, determination of the level of these compounds in vegetables and fruits is consecutively necessary. Therefore, the present study was done with the purpose of measuring nitrite and nitrate contents of tomato used in a tomato paste factory in Kermanshah. Taking samples was done by going to the chosen areas of farms in June and May, and a total of 60 samples were taken and analyzed. Nitrite concentration in tomato samples were determined by spectrophotometric methods at a wavelength of 538 nm, and nitrate concentration was determined after reducing nitrate to nitrite by using cadmium column. Also nitrite and nitrate concentration in irrigation water was determined. The average of nitrite concentration in tomato samples that were measured in north, south, east, west and center plains was equal to 0.74, 0.95, 0.84, 1.14 and 0.9 mg/kg of fresh tomato weight respectively. The average of nitrate concentration in tomato samples that were measured in the mentioned areas was equal to 9.96, 27.11, 13.87, 6.97 and 13.31 mg/kg of fresh tomato weight respectively. According to the results, the average of nitrate concentration in all tomato samples of agricultural areas of Myandarband was less than the WHO standard level. Therefor these products have permissive nitrate level for different applications like producing tomato paste.
Keywords: Nitrite, Nitrate, Tomato, Paste, Kermanshah, Iran

INTRODUCTION

Nitrite (NO_2) and nitrate (NO_3) are as a part of nitrogen natural cycle. These compounds are soluble in water and are composed of nitrogen and oxygen atoms. When N-NH_3 or other forms of nitrogen present in water are aerated, they produce nitrate (Cristiana et al., 2010). The presence of nitrate and nitrite in food is related to vegetable consumption. In previous studies the adverse effects of nitrite on human health have been discussed (Joossens et al., 1996). Using fertilizers with nitrogen contents causes an increase in nitrate concentration of soil. Plants take nitrogen in ionic form of soil (Brownand et al., 1967). Therefore, by increasing the use of fertilizers in farms, nitrate accumulates in the agricultural product (Ximenes et al., 2000). Usually, the nitrite level is much lower than nitrate in vegetables (Aworh et al., 1980). The nitrate concentration rang is naturally about 1-1000 mg/kg of fresh weight (Ximenes et al., 2000). The nitrate content in vegetables may be enhanced depending on different parameters that are related to the plant and environment such as variety, specie, maturity, temperature, amount of some nutrients present in soil and the rate of using fertilizers (Blom-Zandstra et al., 1986). Naturally Nitrate is not toxic for Human; however, the conversion of nitrate to nitrite in human body and its accumulation in high concentrations is very dangerous for the body (Archer, 1989). Presence of nitrite and nitrate in vegetables is attributed to abundant health problems. In digestive tract these substances can react with natural amines and produce carcinogens compounds like N-nitrosamine. The N-nitrosamines that are formed in human stomach are known as a risk factor for stomach, gullet and gallbladder cancers (Bartsch et al., 1990). It is estimated that 80% of cancers are related to environmental factors including water, air and food. On the other hand, dietary, life style and malnutrition directly or indirectly cause 40% of human cancers (Hsu et al., 2009). One of the adverse effects of nitrate is related to combining these compounds with hemoglobin that is present in the blood and leads to reducing nitrate to nitrites, which causes a reduction in potential oxygen transport from lungs to body tissues. Finally, methemoglobinemia may occur (Blue Baby Syndromes) especially in the infants that are under 3 months of age. In older infants and children it may occur too. For example in Spain, several infants aged about 7–13 months experienced methaemoglobinaemia due to consuming vegetables (Chan et al., 2011; Sanchez-Echaniz et al., 2001). In previous studies it was determined that if the amount of nitrite in the vegetables was in high level and it was not properly stored, methaemoglobinaemia could occur in adult's peoples (Zhao et al., 1996). When nitrate amount in drinking water is above 5 mg/L, water is the main source of entering nitrate to the human body. But, when nitrate concentration is lower than 10 mg/L, fruits and vegetables are known as nitrate sources for entering nitrate to the human body (Hsu et al., 2009). According to the data of WHO, adults take 20-70 mg/L N-nitrate by consuming vegetables such as celery, spinage, lettuce, etc. The maximum amount of nitrate that daily enters the body must be less than 3.65 mg/kg (Santamaria et al., 1999). On the other hand, consumption of vegetables and fruits is necessary for human health; because vegetables contain important components of a healthy diet including vitamins, minerals, fibers, etc. their daily consumption helps to prevent different types of diseases such as cardiovascular diseases, cancers and diabetes (Chan et al., 2011; Yordanov et al., 2001). Because of adverse effect of nitrite and nitrate, estimating the level of these compounds in vegetables and fruits is consecutively necessary. One of the agricultural products that has a high level of consumption in Iran is tomato. Until now different investigations have been done on nitrite and nitrate determination in the different material such as vegetables, fruits, meat products and water (Yordanov et al., 2001; Okafor et al., 2003; Ztekin et al., 2002), but there is no study on determination nitrite and nitrate contents of tomato used in tomato paste in Kermanshah. Therefore, the present study was done with the purpose of measuring nitrite and nitrate contents of tomato used in a tomato paste factory in Kermanshah.

MATERIALS AND METHODS

Samples

This study was done in the area between 34 degrees and 22 minutes to 34 degrees and 40 minutes north latitude and 46 degrees and 48 minutes to 47 degrees 13 minutes east of the prime meridian located in the city of Kermanshah, which is one of eleven curacy of Kermanshah province Ghamarnia, 2009). In present study samples took from Myandarband farms in Kermanshah province, by going to the chosen area of farms in June and May. The farms were divided into five regions including north, south, east, west and center, and each region was divided into four parts (fig 1).

Figure1. Myandarband position and sampling station

In any of them three tomato samples were taken and totally 60 samples were taken and analyzed. Taking samples was done randomly at minimum of three times. The samples were transferred to a chemical laboratory of The Faculty of Health in Kermanshah University of Medical Sciences. Then drying procedure was used for measuring nitrite and nitrate contents of tomato samples. The tomato samples were washed with tab water then rinsed with distilled water; next 100 g of them were weighed and stored for 24 h in an oven at 80°C. Then the samples were dried and milled into powder. The experiment was done three times with 2 g of dried tomato weight, and once with 4 g of dried weight. Collected samples were analyzed using Iranian food standard protocols (NO.4106) (Cemek *et al.,* 2007). Five milliliters of sodium tetra-borate ($Na_2B_4O_2$) and 100 cc of warm deionized water (80°C) were added to dry samples and were then incubated for 15 min in a warm water bath. Subsequently, potassium hexacyanoferrate ($K_4Fe(CN)_6$) and Zinc acetate dehydrate ($Zn(CH_3COO)_2$) were added to the solution to produce that content sample. Next this solution was transferred to a 200 ml volumetric flask and diluted with deionized water (Iran Standard, 2010).

Nitrite determination in samples

To determine nitrite, 10 ml of the filtered solution was transferred to a 50 mL volumetric Wings. By adding sulfanilamide chloride and normal 1-naftil ethylene di-amine di-hydrochloride to the filtrated solution, a purple colored complex is produced. Then absorbance was measured at a wavelength of 538 nm using a spectrophotometer (JENOWAY-6715uv/Vis, UKE) (Iran Standard, 2010), and finally the nitrite concentrations in samples were determined by the calibration curve.

Nitrate determination in samples

For determining the amount of nitrate, the filtered solution was mixed with 10 ml of buffer solution (pH=9.6) and cadmium, this step of the experiment causes the reduction of nitrate to nitrite, Samples were diluted to a volume of 50 cc by distilled water. By using 10 ml of this solution the continuance of the experiment was done similar to the nitrite experiment (Iran Standard, 2010).

Analytic method

The amount of nitrite (mg/kg) was calculated according to the following equation (1):

$$NO_2^-(\frac{\text{mg}}{\text{kg}}\ \text{fresh weight}) = \frac{m_1 \times 200}{v_1 \times m_0}) \qquad (1)$$

m_1: mass of nitrite ion in the certain volume of filtered solution obtained from the calibration curve (μg)

v_1: certain volume of filtered solution

m_0: mass of the sample test

And the amount of nitrite (mg/kg) was calculated according to the following equation (2):

$$NO_3^-(\frac{\text{mg}}{\text{kg}}\ \text{fresh weight}) = 1.348(\frac{m_1 \times 10000}{v_3 \times v_2 \times m_1} - \frac{m_1 \times 200}{v_1 \times m_1}) \qquad (2)$$

m_2: total mass of nitrate ion in the certain volume of filtered solution obtained from calibration

v_2: 10 ml of filtered solution test used in spectrophotometer test

v_3: certain volume of filtered solution used for preparation of solution test (Iran Standard, 2010)

Then, the content nitrite and nitrate of tomato samples of five zones were compared using SPSS16. Finally the measured amounts were compared with standard amounts. In all the experiments for diluting solutions distilled water (Ultrapure) was used, and for uniform solutions, micro-centrifuge (MIKRO.120-ZENTRIFUCEN-made in Germane) was utilized.

Nitrite and nitrate determination in irrigation water

For determining nitrite and nitrate content of irrigation water used for irrigating agricultural lands, three samples were collected from each of water well in the 5 regions (north, south, east, west and center) in Myandarband farms. Nitrite concentration in irrigation water was determined using a UV spectrophotometer (DR 5000, HACH Co) according to standard method 2006 and the nitrate concentration was determined after reducing nitrate to nitrite by using cadmium column, (Okafor *et al.,* 2003).

RESULT AND DISCUSSION

The average concentration of nitrite and nitrate in tomato samples in each of the north, south, east, west and center regions in Myandarband agricultural land was investigated and the results are shown in Table 1. Based on the obtained results, the maximum nitrite concentration in tomato samples observed in south and west regions was about 1.24 mg/kg of fresh tomato weight, and the minimum nitrite concentrations observed in the north and south regions was equal to 0.32 and 0.36 mg/kg of fresh tomato weight respectively. Also, for the amount of nitrate in tomato samples, the maximum concentration observed in south region was 39.10 mg/kg of fresh tomato weight, and the minimum concentration in the north region was equal to 3.18 mg/kg of fresh tomato weight.

Table1. The average nitrite and nitrate content of tomato (mg/kg fresh weight) in study regions

Region	Zone	Number	The average nitrite content in tomato (mg/kg fresh weight)	The average nitrate content in tomato (mg/kg fresh weight)
North	1	3	1.12 ± 0.15	11.19 ± 5.01
	2	3	0.36 ± 0.07	3.18 ± 1.25
	3	3	0.32 ± 0.10	11.19 ± 3.79
	4	3	0.42 ± 0.11	4.34 ± 0.91
South	1	3	0.36 ± 0.25	16.92 ± 3.83
	2	3	0.63 ± 0.074	4.44 ± 2.52
	3	3	0.63 ± 0.074	39.10 ± 4.63
	4	3	1.24 ± 0.56	21.89 ± 1.32
East	1	3	1.03 ± 0.21	9.67 ± 5.98
	2	3	0.42 ± 0.22	3.11 ± 1.60
	3	3	0.66 ± 0.043	9.96 ± 2.26
	4	3	0.42 ± 0.22	19.71 ± 6.37
West	1	3	0.57 ± 0.11	6.59 ± 2.90
	2	3	1.15 ± 0.15	5.41 ± 3.23
	3	3	1.24 ± 0.15	4.67 ± 3.16
	4	3	0.48 ± 0.043	4.26 ± 1.75
Center	1	3	0.42 ± 0.34	9.05 ± 4.39
	2	3	0.60 ± 0.11	11.27 ± 5.93
	3	3	1.18 ± 0.26	14.38 ± 1.01
	4	3	0.51 ± 0.043	5.24 ± 2.21

According to the results, nitrate concentration was seen much larger than nitrite concentration in all samples, because the nitrates are stable chemical substances which do not fall under the action of oxidants and temperature; indeed nitrate ions are stable ions (Brownand et al., 1967). Also, vegetables tend to accumulate nitrate ion, especially when using nitrogen fertilizers for more product growth (Zhao et al., 1996). On the other hand, lower concentrations of molybdenum in vegetables cause nitrate in tissues to accumulate; therefore the high concentration of nitrate in vegetables may be a consequence of this (Umah et al., 2003). The average level of nitrate concentration in all tomato samples of agricultural Myandarband land was less than the standard level (300 mg/kg of fresh weight) determined by WHO (WHO, 1978). High standard deviations in some cases may be due to the extent of range of measured nitrate in samples of different areas. The nitrite and nitrate concentration in vegetables are related to two major factors, presence of nitrogen in fertilizer and light intensity that are effective on accumulation of nitrate in vegetables. On the other hand, the extent of nitrate concentration that accumulates in vegetables can be attributed to other factors such as type, species, age of plant, soil nitrate, pH, tension, fertilizer type, frequency and amount of fertilization, cultivation methods (conventional and greenhouse), harvest time (morning or afternoon), harvest season, crop storage after harvesting and climatic conditions (such as temperature and light intensity). In similar studies conducted by others, the extent of nitrate concentration in vegetables has also been reported (Peksa et al., 2006; European commission, 1997).

Obtained results of average nitrite concentrations in five geographic direct of Myandarband plain are shown in Figure 2. The average nitrite concentration in tomato samples that were measured in north, south, east, west and center plains was observed equal to 0.74, 0.95, 0.84, 1.14 and 0.9 mg/kg of fresh tomato weight, respectively. The difference of nitrite concentrations in tomato samples between five geographic regions of Myandarband plain was not significance (P=0.425). In similar studies that were carried out by Ayaz et al to determine the concentration of nitrite and nitrate in vegetables in Turkey, the average nitrate concentration in the tomato samples was equal to 0.36 mg/kg of fresh tomato weight (Ayaz et al., 2007). This amount is less than the obtained amounts in the present study. Another study that was done by Shahlayi et al in Ahwaz to evaluate the content of nitrite and nitrate in vegetables in winter and spring, found that the maximum and minimum nitrite concentration observed in mint (1.51 mg/kg) and in tomatoes (9.45 mg/kg) respectively (Shahlaei et al., 2007), the result of this study reports much higher levels than those obtained results.

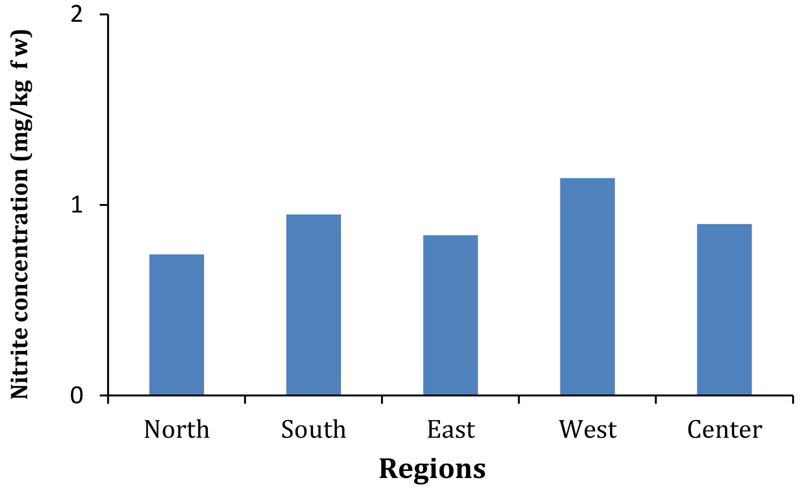


Figure2. Average nitrite content in tomato samples in five regions of Myandarband (mg/kg fresh weight)

The average nitrate concentrations in tomato samples that were collected from each of the five regions of Myandarband plain are shown in Figure 3. According to the results, the average nitrate concentration in the samples that were grown in north, south, east, west and center of plains was observed to be equivalent to 9.96, 27.11, 13.87, 6.97 and 13.31 mg/kg of fresh tomato weight respectively. The difference of nitrate concentrations in tomato samples in south of Myandarband plain was significantly higher than the samples of other regions (p=0.000). This phenomenon can attribute to manual fertilization process that can cause high levels of nitrate to accumulate in the products of this region. Andrei et al in a study that examined the concentrations of nitrite and nitrate in tomatoes and its products show that the average of nitrate concentration in samples was 7.47 mg/kg (Andrei *et al.,* 2012). And in the study that was done by Ayaz et al, the average nitrate concentration in tomato samples was 11.06 ± 13.43 mg/kg of fresh tomato weight (Ayaz *et al.,* 2007), which proves the obtained results of the present study.

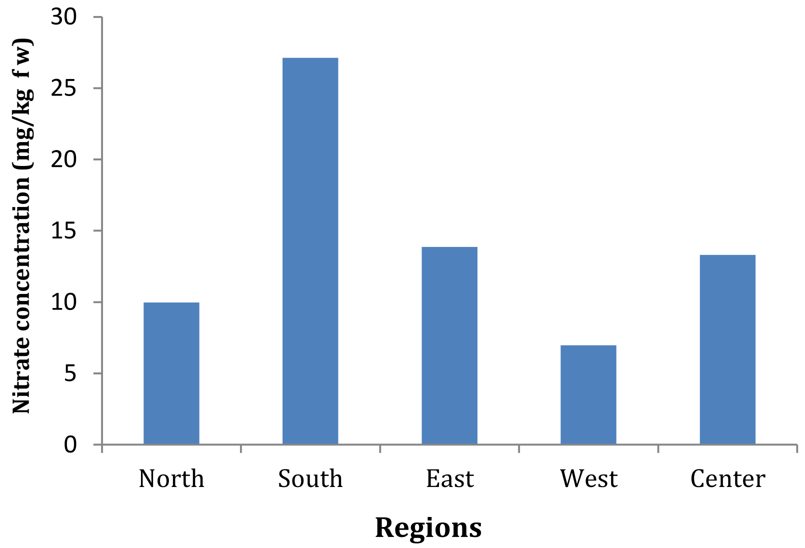


Figure3. Average nitrate content in tomato samples in five region of Myandarband (mg/kg fresh weight)

Based on the average consumption of tomato products in Iran, can estimate the daily intake (DI) of average amounts of nitrate by each person through consumption of tomato and its products (table 2). According to the data of the present study at maximum condition, the daily intake of average amount of nitrate through tomato consumption is 1.35 mg per person that is less than the reported amounts of the study conducted by Santamaria et al (2.77 mg per person) (Santamaria *et al.,* 1999). Therefor these products have suitable levels of nitrate to produce various products like tomato paste.

Table 2. Average consumption and nitrate content and nitrate mean through daily intake of tomato[13]

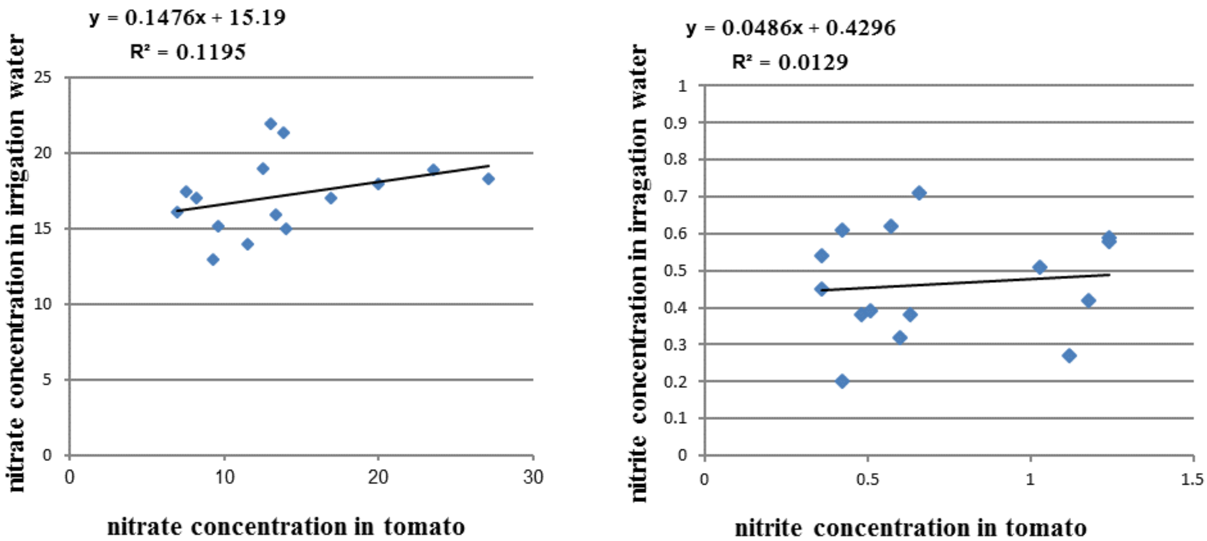
Tomato	Average consumption (g/day per person)	Nitrate content (mg/kg fresh weight)	Nitrate mean daily intake (mg per person)
Minimum	50	9.96	0.49
Maximum		27.11	1.35

Another parameter that affects accumulation of nitrite and nitrate in vegetables is nitrite and nitrate concentration in water used for irrigating this product. In this study, the nitrite and nitrate concentration in the irrigation water that was used to irrigate products in the five areas of Myandarband farmlands was tested and the results are shown in Table 3.

Table 3. The average nitrite and nitrate content in irrigation water in Myandarband farmland (mg/L)

Region	The average of nitrite content in irrigation water (mg/L)	The average of nitrate content in irrigation water (mg/L)
North	0.27 ± 0.02	15.19 ± 2.54
South	0.54 ± 0.11	18.35 ± 4.26
East	0.71 ± 0.15	21.38 ± 3.50
West	0.58 ± 0.14	16.14 ± 3.14
Center	0.32 ± 0.09	15.96 ± 2.07

According to the results, the highest concentration of nitrite in irrigation water was 0.71 mg/L in the eastern farmlands of Myandarband, and the lowest concentration was observed in northern plain equal to 0.27 mg/L. Also for nitrate concentration in the irrigation water, in the eastern plain high concentrations about 21.38 mg/L were observed, and in the northern plain the lowest concentration was observed equal to 15.19 mg/L. As can be seen, despite the differences of nitrite and nitrate concentrations in irrigation water in different parts of the Plain, the total concentration of these compounds in irrigation water was lower than the standard ranges determined for irrigation (10-40 mg/L) and even for drinking water (Rahmani *et al.,* 2006). This could be due to the use of groundwater for irrigation of products in this region. A significant correlation was not observed between the concentrations of nitrite and nitrate in irrigation water and accumulation of these compounds in tomato samples that were tested (fig 4). As mentioned above, the great standard deviation in some cases may be due to the large extent of measured nitrate and manual fertilization process in these areas which can cause large changes in the level of accumulated nitrate in these products.



Figre4. Correlation between concentrations of nitrite and nitrate in irrigation water with accumulation of these compounds in tomato samples

CONCLUSION

The average nitrate concentration in all tomato samples of agricultural Myandarband lands was less than the standard level determined by WHO. Therefore, these products have permissive nitrate levels for different applications like producing tomato paste. The difference of nitrate concentrations in tomato samples in south of Myandarband plain was significantly higher than other regions**.** High standard deviations in some cases may be due to the extent of range of measured nitrate in samples of different areas. Also, a significant correlation was not observed between the concentrations of nitrite and nitrate in irrigation water and accumulation of these compounds in tomato samples that were tested.

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