A survey of Nitrate and Nitrite Contents in Vegetables to Assess The Potential Health Risks in Kurdistan, Iraq

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Abstract

In this study, nitrate and nitrite contents were determined of a total (308) samples of 15 different types of vegetable such as leek, swiss chard, celery, spinach, garden cress, green onion, turnip, radish, aubergine, squash, tomato, pepper, cucumber, mint, and tarragon. They were taken from different fields located in Sulaymaniyyah province: Sulaymaniyyah city (Tanjarro and Kanaswra), Bazyan (Baynjan), Halabja (Said Sadiq), and Kalar (Grda Gozena). Using spectrophotometer measurements performed by UV/VIS double beam spectrophotometer at 538nm. The highest level of nitrate (529.55 mg/kg) for garden cress in Said Sadiq, (486.74 mg/kg) for Swiss chard in Kanaswra, and (477.65 mg/kg) for spinach in Bazyan. In addition, leek and celery contained high nitrate concentrations of about (416.65 and 447.60) mg/kg in Kanaswra and Bazyan, respectively. While the lowest nitrate concentration in fruiting vegetables like a tomato was 5.934 mg/kg in Bazyan, and aubergine in Grda Gozena was 5.617 mg/kg. Interestingly, the value of the Acceptable Daily Intake (ADI) and the Target Hazard Quotient (THQ) was lower than the standard limit, ADI for nitrate in this study was about (0.51, 2.18) mg/kg bw/day for adults and children, while the standard limit was about (3.70 mg/kg bw/day). Also, ADI for nitrite in this study was about (0.01, 0.05) mg/kg/bw for adults and children, while the standard limit was about 0 - 0.06 mg kg, as a result, the THQ <1. In summary, we can conclude that the amount of nitrates in raw vegetables was lower than the standard limit’s level and that this level does not cause health problems for consumers.

Keywords: Vegetables, Nitrate, Nitrogen fertilizers, NPK, Sewage water.

1. Introduction

Nitrate is a natural compound that is part of the nitrogen cycle [1]. With the development of countries, the consumption of vegetables and fruits have been increased. Vegetables are fundamental to human healthiness because they are a kindly source of minerals, biologically active substances, and vitamins. According to the Joint World Health Organization (WHO) that an intake of a lower limit of 400 grams of vegetables and fruits per day should prevent chronic diseases [2]. Since vegetables contain the highest levels of nitrates contributed by foods consumed [3]. Concerns about nitrate intake stem from the fact that it is linked to the formation of some forms of cancer and methemoglobinemia worldwide. In the stomach, nitrate and nitrite are react with secondary amines, and due to the formation of nitrosamines (N-nitroso compounds), which leads to being carcinogenic, mutagenic, and teratogenic. And, this raises the risk of stomach and oesophageal cancer [4]. Nitrites and nitrate are chemicals utilized in fertilizers also can easily transfer from fertilized soil into groundwater. They are produced endogenously in humans and animals. Furthermore, in processed foods, nitrate and nitrite are utilized as preservatives of food like in cured meat products [5].

The plant needs nitrogen for growth while nitrates primary source of nitrogen in the soil. Nevertheless, the nitrate was illustrated as nearly non-toxic, but in the stomach, it changes to the form of nitrite due to reacting with amines as a result of nitrosamines formation [6]. Indeed, high concentrations of nitrate are the global problem in vegetables, the highest rate of nitrate is more than 5000 mg/kg in vegetables particularly leafy vegetables which it reported in various places for instance Mainland China and different countries in Europe [7], [8] and [9]. On the other hand, nitrate exposure might occur via consumption of drinking water, contaminated fish, and proceeded meat food [10]. Dietary nitrates are up taken approximately 80% by consumption of vegetables and fruits [11]. In addition, the factor affecting the concentration of nitrate which is accumulation in vegetables are nitrogen fertilizer [31]. While, it has been verified that these factors are affecting the content of nitrates like genetic factors, lighting ratio, humidity rate, environmental conditions, kind of soil, type of another plant in the field, the harvest time, nitrogen source, storage period, measurement of vegetable, environmental temperature, and degree
of growth plant [12]. On the other hand, vegetables accumulate the highest nitrate concentration and accumulate the lowest amounts of nitrite. Certainly, storage of vegetables after harvest can accumulate the highest concentration of nitrite, due to the reduction of nitrate to nitrite by microbial. According to the European Union Food Commission was established that the Acceptable Daily Intake (ADI) levels of nitrite and nitrate of about 0.06mg/kg Body Weight (BW) and 3.70 mg/kg Body Weight (BW) respectively [12]. Thus, this has led to set regulations regarding the allowable dietary nitrate intake in our food based on the assumption and particularly the amount of nitrate in consumed vegetables by the Kurdish population in Kurdistan. Based on our knowledge, Kurdistan grows a variety of vegetables during the year. However, it is true that in Kurdistan, there is a lack of investigation available for the levels of nitrate concentration in vegetables. Therefore, this study aimed to (i) identifies nitrate and nitrite in various fresh vegetables mostly consumed frequently in Iraqi Kurdistan, (ii) quantify health risks of nitrate and nitrite content from consumption of vegetables.

2. Materials and Methods

2.1. Sampling

The samples of fresh vegetables were collected during July and December of 2020. All chemicals used in this study were analytical grade. Demonized water was used for preparing all the reagents and standard solutions and for sample extraction. All reagents and standards including sodium nitrite (Germany), cadmium sulfate octahydrate (India), hydrochloric acid (Belgium), sodium tetraborate decahydrate, Carrez reagent I (potassium hexacyanoferrate II trihydrate) (Germany), and Carrez reagent II (zinc acetate dihydrate) (India), glacial acetic acid (England), ammonia hydroxide 25% (Spain), and Griess reagent I (sulphanilamide) (Germany) and Griess reagent II (N-(1-naphthyl)-ethylenediamine dihydrochloride) (Germany) was purchased from International Standard ISO (6635: 1984). Zinc in rods was obtained from (Huntingdon Cambridge Limited) and a paper filter (no. 40), both nitrate- and nitrite-free was acquired from Whatman (Germany) in different fields were located in Sulaymaniyah province (See Figure 1).

The equipment used in the research are Spectrophotometric, “Spectrophotometric method mostly depends on the Griess method and are widely utilized in the determination of nitrites and nitrates” [13]. Analytical balance, Boiling Water bath, pH meter, Vertical mechanical agitator, Blender. Where survey sites are indicated. In total (308) samples in 15 different types of vegetables, such as leek, Swiss chard, celery, spinach, garden cress, green onion, turnip, radish, aubergine, squash, tomato, pepper, cucumber, mint, and tarragon were taken during the July until December of 2020. All samples were collected in different locations, which are considered a vegetable source for the Sulaymaniyah region (Tanjarro and Kanaswra), Bazyan (Baynjan), Halabja (Said Sadiq), and Kalar (Grda Gozena) in Kurdistan, Iraq. All analyzes were carried out in six replicates for each vegetable at each site. All vegetables were put in brown paper bags which are stored in a cool box. All samples of vegetables were protected at refrigeration temperature afterwards analyzed within 24h. Finally, all samples of vegetables were analyzed freshly, and the vegetable sample was quickly moving out to the lab for analysis. After that, immediately before analysis, and samples of vegetables were cut into the small portions, and finally, it was homogenized in a GGC-729 Gosonic mixer (Turkey). Recovery experiments were performed using serrated samples with a standard solution of nitrite.

Figure 1. The study areas: Bazyan (Bainjan), Sulaymaniyah (Kanaswra, Tanjarro), Kalar (Grda Gozena), and Halabja (Said Sadiq).
2.2. Extraction

The extraction of nitrite and nitrate was carried out utilizing the Griess diazotization reaction according to International standard [(ISO) 6635: 1984] [14] Griess’s reagent was utilized for measuring nitrite and in the case of nitrate level, a previous reduction of nitrate to nitrite was performed. First, 5 g from sample homogenized has been measured, and it’s going to be put in a beaker of 250 ml. Second, add 50 ml of hot deionized water (70–80 °C), also 2.5 ml of (50 g of Disodium tetraborate in 1000 ml tepid water) solution. Third, the mixture was placed in a water bath with a temperature of (± 100 °C) and heated for 15 min. Next, add 1 ml of (100 g of Potassium hexacyanoferrate and 1000 ml water) solution with 1 ml from (220 g of zinc acetate, 30 ml glacial acetic acid, and in a 1000 ml one- mark volumetric flask and make up to the mark with water) solution. “Then, a sample was transferred to a volumetric flask of 100 ml after cooling to the temperature of the room”. Finally, filtered from a filter paper (Whatman No. 1).

2.3. Determination of nitrate and nitrite

To find out the content of NO₂⁻, 10 ml the sample transferred from the aliquot part of the filtration into 50 ml volumetric flask and add 30 ml deionized water, then add 5 ml of solution (i) (0.4 g of sulfanilamide, 20 ml hydrochloric acid Q₂₀ =1.19 g/ml, and 180 ml hot water). Also, add 3 ml of solution (ii) (445 ml of hydrochloric acid and 555 ml water). At the end add 1 ml of solution (iii) (0.1 g of N-(1-naphthyl) ethylenediamine dihydrochloride and 100 ml water). After adding all solutions were mixed and left at room temperature for 3 min on condition that kept from the light. Makeup to the marker by using water and mix, and it was set in a dark place and measure the absorbance at 538 nm. Although, to determination of nitrate concentration in the samples in 10 ml the sample transfer pipette from the aliquot the aliquot part of the filtration into a conical flask 25 ml and add 2 g of cadmium (zinc rods and cadmium sulfate solution), and add 5 ml buffer solution pH 9.6 (ammonia chloride, water, and ammonia solution Q₂₀ =0.88 g/ml). Stopper the flask and shake for 5 min on the vertical mechanical agitator. Next, filter by using the filter paper, then in the volumetric flask 50 ml collect the filtrate and rinse the filter paper via water several times, after that collect the rinsing water into the volumetric flask, finally make up the marker. To measure the concentration of “total nitrate in the sample the reduction of nitrates to nitrites was accomplished” using. To find out the content of nitrate in 10 ml of the sample transfer via the pipette from the aliquot port of the filtration to a volumetric flask 50 ml and add 30 ml deionized water, then add 5 ml of solution (i). Also, add 3 ml of solution (iii). At the end add 1 ml of solution (ii). After adding all solutions were mixed and left at room temperature for 3 min on condition that kept from the light. Makeup to the marker by using water and mix, and it was set in a dark place and measure the absorbance at 538 nm. Subtract the value for the blank and read the total mass of nitrite from the calibration graph. The blank was prepared in the same method for example instead of the sample replacing via “10 ml of deionized water for the validation the samples were spiked with appropriate volumes of the nitrite standard solutions before extraction” [14]

2.4. Nitrate and nitrite calculation

The nitrate (NO₃⁻) ion content in vegetable extracts was determined depending on (ISO 6635: 1984) utilizing the spectrophotometric method after nitrate reduction and the Griess reaction. While the content of nitrite in vegetable extracts was determined via testing in the absence of the reduction step. In addition, nitrites present in the extracts (both native and resulted from reduced nitrates) were determined by diazotizing with the Griess reagents to form of reddish-purple azo dye that was measured at 538 nm. Spectrophotometric measurements were performed by a UV/VIS double beam spectrophotometer. Utilizing cuvette which was quartz and (1 cm) path length. Nitrate and nitrite quantification was performed using the (calibration standard) method. The calculation of the calibration standard was carried out from regression analysis of (6) standard solutions of nitrites in the range 0, 0.5, 1, 2, 2.5, 3 mg/L. Also, all analyses were carried out in 3 replicates, and the concentration of nitrite was illustrated as (mg/kg) fresh mass depending on Equation (1). The concentration of nitrate was expressed as (mg/kg) fresh mass and was various among the total of content the nitrite after the reduction step three the initial concentration of nitrite depending on Equation (2).

\[
NO₂^- = m₁ \times \frac{200 \times DF}{V₁ \times m₀}
\]

(1)

\[
NO₃^- = 1.348 \times \frac{(m₂ \times 10,000)}{V₂ \times V₁ \times m₀} - \frac{m₂ \times 200}{V₁ \times m₀}
\]

(2)

Where \(NO₂^-\) was the initial concentration of nitrite of the vegetable sample, \(m₀\) = of the test portion is the mass in (grams), \(mₙ\) = is the mass (micrograms) of initial nitrite in the portion filtrate, \(V₁\) = is the read of calibration graph, \(V₂\) = of the test portion was the volume in (ml). And, \(m₂\) in (micrograms) = is the total mass of nitrite ion NO₂⁻ in the portion of the filtrate, \(V₂\) (ml) = of the test solution it has for spectrometric read from the calibration graph, \(Vₙ\) (ml) = of the aliquot portion
of the filtrate taken for the preparation of the test solution. In addition, 1.348 is the proportion among the relative molecular masses of (NO$_3^-$) and (NO$_2^-$) or 1.348 is conversion factor from NO$_3^-$ to NO$_2^-$. Dilution factor (DF), if no dilution carried out the DF=1.

2.5. Risk assessment

The nitrates entering the body out of the vegetable and fruit become a substance of concern, whether it overrides it is Acceptable Daily Intake (ADI) limit or level of toxicity due to it may lead to death [15]. While, the ADI for nitrate dietary was 3.7 mg/kg/BW depending on the regulations of the European Commission’s Scientific Committee on Food (SCF) and Joint Expert Committee of Food and Agriculture (JECFA) [12] [16], [4]. The daily nitrate intake is calculated via assessment of the average daily nitrate accumulation in a person of body-specific weight and also to estimate the relative bioavailability of nitrate. Household Income and Expenditure Survey (HIES) was considered that the average consumption of fruits and vegetables was about 35.5 and 232g respectively [17]. We as well calculate the average Daily Dose (ADDM) and THQ if a person consumes 39, 109, 58, and 39 g/day for onion, tomatoes, lettuce, and leek respectively, in Hamadan province, Iran [18]. In another study in Korean Diet like [19] a person consumed 6.63, 0.40, 7.72, 12.98, 23.24, 1.41, and 0.10 g/day for spinach, swiss chard, radish, cucumber, pepper, aubergine, and celery respectively. The BW for adult of about (70kg) but for children (16.2kg) [20,21]. Depending on the US Environmental Protection Agency (EPA) the Reference Dose (RfD) for nitrate is (1.6) mg nitrate-nitrogen kg/BW/day (equal to approximately 7.0mg nitrate kg/BW/day , while reference Dose for nitrite is 0.1mg/kg/BW/day (equal to approximately (0.33mg) nitrite/ kg/ bw/ day) [22]. The SCF and JECFA have to suggest the ADI for nitrite of about 0–0.07 and 0–0.06mg Nitrite/kg/Bw [23]. The SCF keep that the ADI is viable into each of the source of dietary exposure [12].

2.5.1. Hazard quotient

Non-carcinogenic risks were calculated via the (THQ) mentioned by the previous investigation [24,25]. And (THQ) for assess human health risks from consumption a locally grown product, also THQ was obtained depending on Equation (3).

$$\text{THQ} = \frac{\text{ADDm}}{\text{RfD}}$$

Where:

$$\text{ADDm} = \text{Average daily intake of vegetation} \times \text{concentration of nitrate in vegetables (mg/kg)} / \text{Body weight}$$

Where: ADDm = Average Daily Dose (nitrate)
THQ = Target Hazard Quotient
ADI = Acceptable Daily Intake

If the THQ is rated less than (1), it indicates the acceptable risk level for the chronic health risk, in contrast, but the THQ is higher than (1), which indicated that the risk of non-carcinogenesis is unacceptable [26].

2.6. Statistical Analysis

The data were statistically analyzed according to the method of analysis of variance as a general test. Simple experiment with three replications was used and the data was analyzed with XLSAT program version 7.5.2 and applying Complex Randomized Design (CRD). All possible comparison among the means was carried out by using Duncan’s test at the significant level of 0.05.

3. Result and Discussion

3.1. Comparison of nitrate and nitrite concentration among the sites

3.1.1. Nitrate and nitrite contents in green leafy vegetables

Table 1 shows that there was a significant difference among the results of nitrate contents in garden cress in all four sites, where the highest nitrate content was found in 529.55 mg/kg in Said Sadiq, and 404.73 mg/kg in Bazian, as well as, nitrate content was 439 mg/kg in Kanaswra, and it was 492.68 mg/kg in Grda Gozena. However, there was no significant difference
in nitrate content in Swiss chard between Kanaswra and Grda Gozena which were about 417.44 and 411.19 mg/kg, respectively. In addition, the wastewater has been used for irrigation in Kanaswra, and ordinary water in Grda Gozena, this might be due to the high application of fertilizer in the Grda Gozena. While, the different locations (Bazyan and Said Sadiq) show a significant effect on the nitrate content in Swiss chard which were 486.74 and 244.59 mg/kg, respectively. These different results related to the method of vegetable breeding in Bazian a high application of nitrogen fertilizer for cultivation, but, nitrogen fertilizer was not applied in Said Sadiq. While at two locations, Swiss chard breeds in the open field and ordinary water is used for the irrigation. Overall, the results showed that the highest content of nitrate in the Swiss chard 486.74 mg/kg was found in Bazyan. The amount of nitrate in spinach samples was differed significantly among all sites and ranged from 477.65, 418.79, 254.12, and 207.44 mg/kg in Bazyan, Kanaswra, Garda Gozena, and Said Sadiq, respectively. However, the nitrate levels generally were below the allowable range established by the World Health Organization (WHO), which it showed in table 4 [27], and in the Korean survey 4259mg/kg [28]. The nitrate and nitrite contents was a significant difference in leeks between two locations (Kanaswra and Garda Gozena) 416.65 and 126.14 mg/kg, respectively, and these results were higher compared to nitrate content of leeks were 62.95 and 54.13 mg/kg in Bazyan, Said Sadiq, respectively, which was a slight difference between these two locations. This result is lower than the results of the previous investigation, for instance, leeks in Iran were about (1070.79 mg/kg) [18]. The nitrate level in leeks was very low in Bazyan and Said Sadiq compared to Kanaswra and Garda Gozena due to the NPK fertilizer has not been applied by the farmers and only ordinary water used for the irrigation. These nitrate levels in leeks were lower compared to the previous study conducted in Iran 1070.79 mg/kg [18].

In other words, green leafy vegetables including celery was a significant difference among 447.60 mg/kg in Bazyan and 404.18 mg/kg in Kanaswra. On the other hand, there was no significant difference between the nitrate content in celery 27.75 mg/kg and 55.07 mg/kg in Said Sadiq and Grda Gozena, respectively, as the young celery leaves have been used for nitrate analysis which is following the results reported by (Greenwood and Hunt, 1986) [29] that older leaves had a higher concentration of nitrate compared with young leaves. Explain such a relation by cabbage which has a smaller concentration of nitrate in the innermost leaves compared with the outer leaves contain the highest concentrations of nitrate, additionally, celery in Said Sadiq were young leaves and it contained low concentrations of nitrate. Various parameters can be affected the concentration of nitrate in between sites as mentioned. The comparatively high level of nitrate concentration in celery may be concerning to the climatologic condition like lower light intensity because of the time of sampling in Said Sadiq at the end of November. Which was considered a success factor in the accumulation of nitrate [30]. Overall, green leafy vegetables were contained the highest level of nitrate compared with other vegetables analysed in table 1.

| Vegetable | Location | Sample NO | Mean NO$_3$ | Mean NO$_2$
|-----------|----------|-----------|-------------|-------------|
| Swiss chard | Kanaswra | 6 | 417.44b | 1.24b |
| | Grda Gozena | 6 | 411.19b | 0.22c |
| | Bazyan | 6 | 486.74a | 0.58c |
| | Said Sadiq | 6 | 244.59c | 3.55a |
| spinach | Kanaswra | 6 | 418.79b | ND |
| | Grda Gozena | 6 | 254.12c | 3.74a |
| | Bazyan | 6 | 477.65a | 0.05b |
| | Said Sadiq | 6 | 207.44d | 3.51a |
| Garden cress | Kanaswra | 6 | 439.00c | 0.07d |
| | Grda Gozena | 6 | 492.68b | 3.98b |
| | Bazyan | 6 | 404.73d | 6.26a |
| | Said Sadiq | 6 | 529.55a | 1.63c |
| Leek | Kanaswra | 6 | 416.65a | 2.55a |
| | Grda Gozena | 6 | 126.14b | 0.19c |
| | Bazyan | 6 | 62.95c | 0.20c |
| | Said Sadiq | 6 | 54.13c | 1.88b |
| Celery | Kanaswra | 6 | 404.18b | 2.80a |
| | Grda Gozena | 6 | 55.07c | 0.03c |
| | Bazyan | 6 | 447.60a | 0.38b |
| | Said Sadiq | 6 | 27.75c | 0.08c |

Table 1. Nitrate and nitrite contents in green leafy vegetables(mg/kg FW).
3.1.2. Nitrate and nitrite content in fruiting vegetables

As presented in table (2) the nitrate content in fruiting vegetables were the lowest at all sites compared to the other group of vegetables (green leafy vegetables and herbs and tuber vegetables). However, the results showed a significant difference among the samples of fruit vegetables, where the nitrate content in aubergine had the highest level of nitrate 115.22 mg/kg in Kanaswra and the lowest level was 5.62 mg/kg in Grda Gozena, this might be due to the high application of nitrogen fertilization by the farmers in Bazyan while ordinary water has been used for the irrigation at both sites (Bazyan and Grda Gozena). Also, this might be due to different environmental conditions (i.e. duration of sun exposure and sowing time). The average nitrate content in tomato fruits was 16.98 mg/kg in Said Sidiq, and this result is in line with the nitrate content in tomatoes with an average of 16.95 mg/kg [38]. As well, it was higher compared to Bazyan and Grda Gozena, reaching 5.93 and 6.24 mg/kg, respectively. The chicken manure has been used as a source for nitrogen in Bazyan, while any fertilizers have not been used in Grda Gozena.

The average nitrate content in squash was a significantly different value in all sites about 91.82, 63.42, 25.25 mg/kg in Bazyan, Said Sadiq, and Garda Gozena, respectively. Together, the nitrate contents in peppers were 33.69, 26.32, and 6.24 mg/kg in Bazyan, Said Sadiq, and Grda Gozena, respectively. In contrast, in cucumbers 24.16, 17.56, and 12.87 mg/kg, in Bazian, Said Sadiq, and Grda Gozena, respectively. Pepper had a lower level of nitrate compared to a previous study conducted in Beijing, China which contained 218 mg/kg [9].

As presented in table (4) the average nitrate content in fruiting vegetables were lower compared to the previous investigations. However, all the fruiting vegetables were collected during the summer season between July and September. But the content of nitrate and nitrite in cold season vegetables was higher than in warm season [32]. Biological factors are responsible for nitrate accumulation in the mesophyll cells of the leaves; fruits and seeds have low nitrate levels because nitrate is exclusively transported by the xylem. In addition, the vegetable organs can be listed in the decreasing order of the nitrate content as follows: petiole>leaf>stem>root>inflorescence>tuber>bulb>fruit>seed [33,34] and [35].

| Vegetables  | Locations | Sample No | Mean NO$_3$ | Mean NO$_2$
|-------------|-----------|-----------|-------------|-------------
| Aubergine   | Kanaswra  | 6         | 115.22 a    | 0.26 c      |
|             | Grda Gozena | 6         | 5.62d       | 0.56 d      |
|             | Bazyan    | 6         | 53.92 b     | 3.39 a      |
|             | Said Sadiq | 6         | 43.06 c     | 2.70 b      |
| Pepper      | Grda Gozena | 6         | 6.24 c      | 0.20 c      |
|             | Bazyan    | 6         | 33.69 a     | 0.91 a      |
|             | Said Sadiq | 6         | 26.32 b     | 0.56 b      |
| Cucumber    | Grda Gozena | 6         | 12.87 c     | 0.07 b      |
|             | Bazyan    | 6         | 24.16 a     | 0.29 b      |
|             | Said Sadiq | 6         | 17.56 b     | 2.10 a      |
| Tomato      | Grda Gozena | 6         | 6.24 b      | 0.20 b      |
|             | Bazyan    | 6         | 5.93 b      | 0.03 c      |
|             | Said Sadiq | 6         | 16.98 a     | 1.72 a      |
| Squash      | Grda Gozena | 6         | 25.25 c     | 0.09 b      |
|             | Bazyan    | 6         | 91.82 a     | 2.20 a      |
|             | Said Sadiq | 6         | 63.42 b     | 2.45 a      |

3.1.3. Nitrate and nitrite content in herbs and tuber vegetables

From (Table 3) which given an overview of nitrate and nitrite contents in herbs and tuber vegetables including mint, tarragon, and green onion, there was a significant difference of nitrate content in mint between Kanaswra and Grda Gozena which were 90.28 and 47.87 mg/kg, respectively. It was lower compared to another study conducted in Iran and which they found 684 mg/kg in mint [36].
On the other hand, the amount of nitrate in tarragon was significantly different in the two locations of Bazyan and Kanaswra which were 353.29 mg/kg and 47.10 mg/kg, respectively. While the amount of nitrate in tarragon in Bazyan was higher compared to a study conducted in Iran 83 mg/kg [36]. This is due to environmental and agricultural factors that can influence the nitrate contents in vegetables. As well as, soil moisture, light intensity, temperature and the latter fertilizers, variety and crop protection strategies [37]. The nitrate content in green onions differed significantly among samples in all sites, the highest level was in Bazyan 82.77 mg/kg compared to the lowest was 27.68 mg/kg in Said Sadiq. Regarding the root or tuber vegetables, there was not a significant difference of nitrate level in radish between Grda Gozena and Bazyan which were 229.69 and 224.02 mg/kg, respectively, but these results were significantly different compared with Kanaswra and Said Sadiq which were 61.93 and 42.10 mg/kg, respectively. The levels of nitrate found in radish surveyed in this study showed that the results are below the levels reported in similar products grown elsewhere in the world, especially in Xiamen, China which was 2078 mg/kg [9]. Moreover, nitrate accumulation in turnip was a significant difference among (367.46, 170.87, 157.62, and 28.34) in Kanaswra, Bazyan, Grda Gozena, and Said Sadiq, respectively. In Said Sadiq, nitrogen fertilizer has not been applied by the farmers, and ordinary water has been used for irrigation. In contrast, nitrogen fertilizer has been applied in Kanaswra. The results of our investigations showed that tables (1), (2) and (3) the content of nitrates in samples of vegetables was less than the maximum level for nitrates as recommended by the WHO which showed in the table (4) this means that the vegetables are safe for consumption.

### 3.1.4. Nitrite content in different vegetables surveyed at different locations

The average nitrite content of all vegetable types at all locations was illustrated in tables (1, 2 and 3). The nitrite content in garden cress and turnip in Bazyan was about 6.26 and 4.00 mg/kg, respectively, and they were higher compared to spinach samples were not detected (ND) mg/kg in Kanaswra and 0.05 mg/kg in Bazyan, in addition, radish was 0.02 mg/kg in Kanaswra, green onion was 0.02 mg/kg in Said Sadiq, celery was 0.03 mg/kg in Garda Gozena, and garden cress was 0.07 mg/kg in Kanaswra, cucumber 0.06 was mg/kg in Grda Gozena, and turnip 0.08 mg/kg in Kanaswra, due to the nitrite contents in vegetables were generally below the allowable range. While in another study, the average nitrite content in tomatoes was 0.13 and 0.16 mg/Kg, with a range of 0.01 - 0.37 and 0.04 - 0.28 mg/kg for different locations, and cucumber was almost 0.15 - 8.05 mg/kg at Tulkarm district in Palestine [38]. However, the results for the nitrite content in this survey were less than 1 mg/kg except for several samples which were above 1 mg/kg, but generally, the concentration of nitrite was under the allowable range and according to [40]. Which must be under than (1mg/Kg) but still in an acceptable range and propose no danger to human health. It was reported that the nitrite levels start to be dangerous if it is higher than 100 mg/Kg [39]. Based on these data, nitrite contents of vegetables at all locations are low and no need to be concerned. The nitrite content in vegetables are usually very low but after the harvest nitrate will be converted into nitrite and which it is relatively slow, while the assimilation proceeds fairly rapidly as a result the content of nitrite of vegetables are not higher than (1-2) mg/kg in fresh product[29].

| Vegetable     | Location     | Sample NO | Mean NO$_3$ | Mean NO$_2$ |
|---------------|--------------|-----------|-------------|-------------|
| Mint          | Kanaswra     | 6         | 90.28a      | 1.94a       |
|               | Grda Gozena  | 6         | 47.78b      | 0.16b       |
| Tarragon      | Bazyan       | 6         | 353.29a     | 3.25a       |
|               | Kanaswra     | 6         | 47.10b      | 0.51b       |
| Green onion   | Kanaswra     | 6         | 35.89c      | 3.22a       |
|               | Grda Gozena  | 6         | 64.39b      | 2.11b       |
|               | Bazyan       | 6         | 82.77a      | 0.28c       |
|               | Said Sadiq   | 6         | 27.68c      | 0.02d       |
| Radish        | Kanaswra     | 6         | 61.93b      | 0.02d       |
|               | Grda Gozena  | 6         | 229.69a     | 4.58a       |
|               | Bazyan       | 6         | 224.02a     | 0.44c       |
|               | Said Sadiq   | 6         | 42.10b      | 2.46b       |
| Turnip        | Kanaswra     | 6         | 367.46a     | 0.08b       |
|               | Grda Gozena  | 6         | 157.62b     | 0.31b       |
|               | Bazyan       | 6         | 170.87b     | 4.00a       |
|               | Said Sadiq   | 6         | 28.34c      | 3.89a       |
Table 4. Nitrate concentration in the samples in our study compared with WHO limit [27].

| Vegetables | Nitrate (mg/kg) | Standard Limit (mg/kg) |
|------------|-----------------|------------------------|
| Dill       | 524.14          | 2000                   |
| Spinach    | 207.44-477.65   | 2000-3000              |
| Tarragon   | 47.09-353.29    | -                      |
| Leek       | 54.13-416.65    | 2000-2500              |
| Lettuce    | 131.82          | 2000                   |
| Mint       | 47.78-90.27     | 3000                   |
| Celery     | 27.74-447.60    | -                      |
| Cress      | 404.09-529.55   | -                      |
| Pepper     | 6.24-33.69      | 200                    |
| Cucumber   | 12.87-24.15     | 150                    |
| Tomato     | 5.9-16.9        | 300                    |
| Eggplant   | 5.61-115.22     | 400                    |
| Turnip     | 28.34-367.46    | -                      |
| Onion      | 27.76-82.76     | 80                     |
| Swiss chard| 244.58-486.73   | -                      |
| Radish     | 42.09-229.69    | -                      |
| Squash     | 25.25-91.82     | -                      |

Table 5. The levels of the nitrate obtained in this study were compared with previous studies.

| Vegetables | NO₃ in this work (mg/kg) | NO₃ in other study(mg/kg) | Countries | References |
|------------|--------------------------|---------------------------|-----------|------------|
| Swiss chard| 486.74                   | -                         | -         | -          |
| Spinach    | 477.65                   | 501                       | Iran      | [36]       |
| Garden cress| 529.55                | -                         | -         | -          |
| Aubergine  | 115.223                  | 350                       | Hong Kong, China | [9]        |
| Pepper     | 33.692                   | 218                       | Beijing, China5 | [44]      |
| Cucumber   | 24.158                   | 212                       | Korea     | [28]       |
| Tomato     | 16.977                   | 16.955                    | Tulkarm District | [9]       |
| Squash     | 91.819                   | 654                       | Korea     | [36]       |
| Leek       | 416.65                   | 191                       | Iran      | [36]       |
| Celery     | 447.60                   | 1103                      | European Countries |          |
| Mint       | 90.28                    | 684                       | Iran      | [36]       |
| Tarragon   | 353.29                   | 83                        | Iran      | [36]       |
| Green onion| 82.77                    | 164                       | European Countries | [9]       |
| Radish     | 229.69                   | 2078                      | Xiamen, China | [9]       |
| Turnip     | 367.46                   | -                         | -         | -          |
| Lettuce    | 131.83                   | 950                       | Hong Kong, China | [9]       |
| Dill       | 524.15                   | 772                       | Iran      | [36]       |
3.2. Acceptable daily intake (ADI)

The result showed in tables (6) and (7). The Leek sample had the highest ADI value (0.232mg/kg) for adults, but for children, it was around 1.003mg/kg due to children having less weight, which is 16.2 kg. While, the lettuce, tomato, onion, spinach, Swiss chard, radish, cucumber, pepper, aubergine, and celery samples had the ADI value for adult (0.109, 0.026, 0.046, 0.045, 0.003, 0.025, 0.004, 0.011, 0.002, and 0.001) mg/kg, at the same time for children were approximately 0.472, 0.114, 0.199, 0.195, 0.012, 0.109, 0.019, 0.048, 0.010, and 0.003 mg/kg respectively. ADI for leek, lettuce, tomato and onion were amount 0.60, 0.89, 0.17, and 0.05 mg/kg respectively in Hamadan province, Iran [18]. Total daily intake nitrate for adults and children were lower about 0.51 and 2.18 mg/kg compared with the ADI (3.70 mg/kg bw/day) [12,16]. Total daily intake nitrite for adults and children were lower approximately (0.01 and 0.05) mg/kg compared with the ADI of 0–0.06 mg/kg for nitrite [12]. The weekly nitrate intake has been estimated via a member of the general population roughly in the USA or England to average approximately (400-500 mg) while these figures cannot generally be applied due to differences in feeding habits and concentrations of nitrate in water and food [39]. In the body, nitrate will be converted to nitrite and leads to the formation of a toxic compound. As a result, in the stomach forming compound namely N-nitrosamines and it leads poses like in many tissues leads to stimulation of cancer (i.e. the lung, kidney, brain, colon, pancreas, oesophagus, tongue, and bladder [41]. Depending on the European Commission’s Scientific Committee on Food (SCF) and the Joint Expert Committee of Food and Agriculture (JECFA), in between daily intake and degree of toxicity of nitrate have a direct correlation, and the (ADI) for dietary nitrates was about 3.7 mg/kg BW [12,42]. The Acceptable daily intake (ADI) and total hazard quotients (THQ) results for nitrate and nitrite were demonstrated in tables (6) and (7). While, daily exposure was about 62.21mg/kg for lettuce, leek 41.76mg/kg, tomato 12.09mg/kg and 3.21mg/kg for onion according to this study. On the other hand, daily exposure was about (0.44, 0.04, 0.03, 0.05, and 0.08) mg/kg for spinach, Swiss chard, radish, cucumber and pepper respectively [19].

3.3. Non-carcinogenic risk

There are a lot of factors affecting hazards of health linked with nitrate for example frequency of consumption, BW of consumption, and the essential risk caused via nitrates. Besides The health risks associated with vegetable consumption are shown in table (6). And in the study, the values of THQ for all samples were under 1, Hence, the population in Sulaymaniyah city can consume these types of vegetables due to the THQ is less than 1 of all kinds of vegetables. As well, the values THQ obtained for nitrate samples like lettuce, tomato, leek, onion, spinach, Swiss chard, radish, cucumber, pepper, aubergine and celery were 0.29, 0.07, 0.63, 0.12, 0.12, 0.01, 0.07, 0.01, 0.03, 0.01, and zero for children respectively. On the other hand, the values of THQ obtained for nitrite samples like lettuce, tomato, leek, onion, spinach, Swiss chard, radish, cucumber, pepper, aubergine, and celery were 0.13, 0.12, 0.06, 0.08 0.02, 0.00, 0.02, 0.02, 0.01, 0.00, and 0.00 for children, respectively.

Table 6. Acceptable daily intake and total hazard quotients of nitrate from consumption of various vegetables.

| Vegetables   | NO3 con. | DI  | Daily exposure | ADIa | ADIb | THQa | THQb |
|--------------|----------|-----|----------------|------|------|------|------|
| Onion        | 82.77    | 0.04| 3.21           | 0.046| 0.199| 0.03 | 0.12 |
| Tomato       | 16.98    | 0.11| 12.09          | 0.026| 0.114| 0.02 | 0.07 |
| Lettuce      | 131.83   | 0.06| 62.21          | 0.109| 0.472| 0.07 | 0.29 |
| Leek         | 416.65   | 0.04| 41.76          | 0.232| 1.003| 0.15 | 0.63 |
| Spinach      | 477.65   | 0.01| 0.44           | 0.045| 0.195| 0.03 | 0.12 |
| Swiss chard  | 486.65   | 0.00| 0.04           | 0.003| 0.012| 0.00 | 0.01 |
| Radish       | 229.69   | 0.01| 0.03           | 0.025| 0.109| 0.02 | 0.07 |
| Cucumber     | 24.16    | 0.01| 0.05           | 0.004| 0.019| 0.00 | 0.01 |
| Pepper       | 33.69    | 0.02| 0.08           | 0.011| 0.048| 0.01 | 0.03 |
| Aubergine    | 115.22   | 0.00| 0              | 0.002| 0.010| 0.00 | 0.01 |
| Celery       | 477.65   | 0.00| 0              | 0.001| 0.003| 0.00 | 0.00 |

NO3 con= Mean NO3 content mg/kg, DI= Daily consumption (g/day), Daily exposure mg/kg, ADIa= mg/kg/bw/day for Adult, ADIb= mg/kg/bw/day for Children, THQa= total hazard quotients for adult, THQb= total hazard quotients for children. TDI (total daily intake) NO3 = 0.51 for adult, 2.18 for children.
Table 7. Acceptable daily intake and total hazard quotients of nitrite from consumption of various vegetables.

| Vegetables       | NO2 con. | DI   | Daily exposure | ADDa | ADDb | THQa | THQb |
|------------------|----------|------|----------------|------|------|------|------|
| Onion            | 3.22     | 0.04 | 3.21           | 0.002| 0.008| 0.02 | 0.08 |
| Tomato           | 1.72     | 0.11 | 12.09          | 0.003| 0.012| 0.03 | 0.12 |
| Lettuce          | 3.731    | 0.06 | 62.21          | 0.003| 0.013| 0.03 | 0.13 |
| Leek             | 2.55     | 0.04 | 41.76          | 0.001| 0.006| 0.01 | 0.06 |
| Spinach          | 3.74     | 0.01 | 0.44           | 0.000| 0.002| 0.00 | 0.02 |
| Swiss chard      | 3.55     | 0.00 | 0.04           | 0.000| 0.000| 0.00 | 0.00 |
| Radish           | 4.58     | 0.01 | 0.03           | 0.001| 0.002| 0.01 | 0.02 |
| Cucumber         | 2.10     | 0.01 | 0.05           | 0.000| 0.002| 0.00 | 0.02 |
| Pepper           | 0.91     | 0.02 | 0.08           | 0.000| 0.001| 0.00 | 0.01 |
| Aubergine        | 3.39     | 0.00 | 0              | 0.000| 0.000| 0.00 | 0.00 |
| Celery           | 2.80     | 0.00 | 0              | 0.000| 0.000| 0.00 | 0.00 |

NO2 con. = MeanNO2content mg/kg, DI= Daily consumption (g/day), Daily exposure mg/kg, ADDa= mg/kg/bw/day for Adult, ADDb= mg/kg/bw/day for Children, THQa= total hazard quotients for adult, THQb = total hazard quotients for children. TDI (total daily intake) NO2 = 0.01 for adult, 0.05 for children

Conclusion

Vegetables are the major source of dietary nitrate intake of humans and nitrate has many detrimental and some beneficial effects on human health. This survey was performed to assess nitrate and nitrite concentrations in several vegetables at four different locations in Iraqi Kurdistan. This study has produced results demonstrating that the nitrate and nitrite contents in vegetables are similar to those reported by WHO and some of the data were even found lower than those of the WHO, implying the non-toxicity of these vegetables. In addition to assessing ADI and health risks with the aid of THQ. In Kurdistan, for the first time a survey of the concentration nitrate and nitrite obtained from vegetables, but it is not known how many vegetables have been eaten per day and the amount of nitrate exposure to the body, so we relied on other research regarding ADI and THQ. This study obviously explains that the mean nitrate and nitrite concentration in almost all samples was lower compared with the standard level. The nitrate concentrations were higher in green leafy vegetables and stem vegetables than herbs and tuber or root vegetables and fruiting vegetables. Therefore, the intake of nitrate through vegetables could be considered safe for consumers. Due to Acceptable daily intake (ADI) and (THQ) and total hazard quotients of nitrate and nitrite from consumption of various vegetables were lower than the standard limit.

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