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# NITRATE IN RAW AND COOKED VEGETABLES: CONTENT, VARIATION AND INFLUENCE OF COOKING METHODS

**Al_Nawaiseh FY[1] and RH Mashal[2]***

**Faten Yousef Al_Nawaiseh**

*Corresponding author email: rima@ju.edu.jo
ORCID ID: https://orcid.org/0000-0002-2602-1841 - Mashal RH

[1]Faten Yousef Al_Nawaiseh: The PhD candidate; Department of Nutrition and Food Technology, Faculty of Agriculture, University of Jordan. Jordan Food and Drug Administration

[2]Rima Hussein Mashal, Associate Professor of Experimental Nutrition and Dietetics, Head of Department of Nutrition and Food Technology, Faculty of Agriculture, University of Jordan. P.O. Box 13701, Amman, 11942 Jordan

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## ABSTRACT

Nitrate concentrations in vegetables are pivotal in dietary nitrate benefit-risk assessments. This study examined nitrate and nitrite levels in vegetables commonly consumed in Jordan, highlighting the impact of different cooking methods (boiling, steaming, baking, stir-frying, and deep frying) on nitrate content. The study examined the nitrate and nitrite levels in 270 raw vegetables and vegetable-based dishes purchased from retail markets and restaurants in Amman, Jordan, during the winter months of 2022 and 2023. Furthermore, 91 experimentally -cooked vegetables were tested for their nitrate and nitrite contents before and after cooking to determine the effect of cooking methods on nitrate levels. The nitrate and nitrite concentrations were measured using an in-house validated molecular absorption spectrometric method. Nitrate levels varied considerably within and between vegetable groups and species. Celery had the highest mean nitrate content (1700 mg/kg), and radish (1300 mg/kg) and beetroot (900 mg/kg) were next. Approximately 32% of vegetables contained moderate amounts of nitrate (200–1000 mg/kg). Leafy greens such as spinach, tossa jute, and lettuce contain nitrates in averages exceeding 500 mg/kg. Among fruit vegetables, zucchini and eggplant have the highest mean nitrate levels (311.6 and 221.6 mg/kg, respectively). Flower, root (excluding radishes and beetroot), and legume (excluding green beans) vegetables have nitrate levels below 200 mg/kg. On average, the mean nitrite level in raw and cooked vegetables was less than 1 mg/kg. Different cooking methods influence vegetable nitrate content differently. Boiling reduced nitrate by 22-40%. Steaming had no significant impact. Although deep-frying, stir-frying, and baking increased nitrate by 130%, 66.7%, and 57.6%, respectively, the overall effect was not statistically significant for deep-frying and baking. These findings are essential for public health and food safety authorities in evaluating nitrate exposure and assessing the risk and benefit of dietary nitrate intake. The data may support the development of evidence-based guidelines for safe nitrate intake.

**Key words:** Nitrate, nitrite, raw vegetables, cooked vegetables, cooking methods

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## INTRODUCTION

Vegetables are excellent sources of vitamins, minerals and dietary fiber [1]. Its consumption is associated with lower risks of cardiovascular disease, cancer and mortality [2]. The World Health Organization (WHO) recommends consuming at least 400 g of vegetables daily [1]. The 2020–2025 American Dietary Guidelines recommend including a wide range of vegetables to ensure nutrient variety [3].Vegetables are also a significant source of nitrates, accounting for 80% of dietary nitrate intake [4, 5].

Nitrate is a natural constituent of plant-based foods as part of the nitrogen cycle and is widely distributed throughout the environment[6]. Nitrate in food was long considered a contaminant before its beneficial effects were clarified [5-7]. Food safety bodies like the European Food Safety Authority (EFSA) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) have extensively reviewed nitrate and nitrite safety. They concluded that nitrate itself has low toxicity, but dietary nitrate poses risks when converted into nitrite [8, 9]. Nitrite is toxic because of its potential to form N-nitroso compounds and is associated with gastrointestinal cancer. It can also cause infantile methemoglobinemia, which impairs blood oxygen transport [10].

The International Agency for Research on Cancer (IARC) classifies nitrate and nitrite as Group 2A carcinogens, which are probably carcinogenic to humans when ingested under conditions that promote endogenous nitrosation [6].To protect consumer health, many European countries have established maximum allowable nitrate levels in vegetables, especially leafy greens [9]. Food safety organizations, including JECFA and EFSA, have set acceptable daily intake levels at 0–3.7 mg/kg body weight for nitrate and 0–0.6 mg/kg for nitrite [8, 9]. However, dietary nitrate also plays a beneficial role by enhancing nitric oxide bioavailability through the nitrate-nitrite-nitric oxide pathway, supporting cardiovascular and endothelial health [7, 11].

Nitrate levels in plant-derived foods vary widely and are affected by factors such as plant genetics, agricultural practices, and environmental conditions such as drought, light and temperature [12, 13]. Cooking could modify a vegetable's chemical composition, affecting nutrient and contaminant concentration [14]. Numerous studies have examined the impact of different cooking techniques on vegetable nitrate levels; nevertheless, the results were not consistently uniform [15-19]. Boiling vegetables generally reduced nitrate concentrations at varying rates [15-18], some of which were insignificant [19, 20]. In contrast, frying yields variable outcomes for the same vegetable type. For instance, some studies reported elevations in nitrate levels in potatoes after deep frying [15-17], while others indicated reductions [19, 21]. Moreover, each vegetable type may respond differently to the same cooking

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method; for instance, stir-frying spinach elevated nitrate levels, whereas cabbage remained unaffected [20]. These varied outcomes suggested that the interaction between specific cooking methods and vegetable types could determine the impact of cooking on nitrate levels.

No comprehensive database exists for the nitrate levels of plant-based foods in Jordan. The published research regarding nitrate levels in vegetables from Jordan is limited, with these studies primarily aimed at identifying gaps in agricultural practices concerning nitrate content [22, 23]. A nitrate database is crucial for performing risk-benefit analyses of dietary exposure to nitrates, supporting public health guidelines and regulatory standards, and improving agricultural practices. This study aimed to create a reference database of nitrate and nitrite levels in commonly consumed vegetables in Jordan. The study also examined the impact of various cooking methods on the nitrate content of selected fresh vegetables.

## MATERIALS AND METHODS

### Sampling and Samples Preparation

Fresh vegetables and vegetable-based dishes commonly consumed in Jordan were purchased from retail markets and restaurants in Amman City between December 2022 and April 2023. Most vegetables in Amman are supplied by the central horticultural market, which is managed by the Greater Amman Municipality. The winter season was chosen for sampling to capture peak nitrate levels, as colder months typically show higher nitrate content in vegetables than in summer. Vegetables were rinsed with tap water to remove adhered dirty particles, drained with absorbent paper or a towel, and removed from the inedible portion. Before analysis, the samples were chopped, blended in a blender, labeled and frozen at -18°C in plastic polyethylene bags. The vegetable-based dishes included in this study reflect the regional food culture [24, 25]. These dishes include tabbouleh, a salad of parsley, tomatoes, and bulgur; coleslaw, a shredded cabbage salad with dressing; hummus, made from chickpeas, tahini, and lemon; falafel, deep-fried patties of chickpeas and fava beans and fulmedames, stewed fava beans with lemon, garlic and olive oil.

### Cooking Process

A subset of fresh vegetables was selected to measure raw and cooked nitrate and nitrite contents to examine the effects of various domestic cooking techniques on nitrate levels. Vegetable selection for each cooking method was based on traditional cooking practices followed in Jordan and the Middle East [24, 25]. Five cooking methods were selected: boiling, steaming, baking, stir-frying and deep-frying, adhering to procedures similar to typical household practices.

To boil, broccoli and cauliflower florets, unpeeled carrot slices (1/2-inch-thick), pit-sized green beans (1-2 inches), and unpeeled beetroots and potatoes were placed separately in boiling tap water at a ratio of 2:1, water to vegetables. The estimated cooking durations were as follows: 10 min for broccoli and cauliflower, 10-12 min for carrots and green beans, and 15-20 min for beetroots and potatoes. To steam, cauliflower florets, unpeeled carrots and zucchini slices (1/2-inch-thick) were arranged in a steam basket above boiling water in a covered pot for 10 min for the carrots and cauliflower and 5 min for the zucchini until they were tender.

For baking, round slices of zucchini, peeled potatoes, and eggplant (1/4 to 1/2-inch-thick) were lightly coated with olive oil and baked in an oven for 20–25 min at 180°C. For stir-frying, pit-sized green beans (1-2 inches), spinach, and mallow were added separately to a heated pan containing olive oil for 10 min until tender. For deep-frying, cauliflower florets, long thin peeled potato sticks (1/4 to 1/2 inch in width and 3-4 inches long), and round peeled eggplant slices (1/4 to 1/2-inch-thick) were each dipped separately in heated sunflower oil at 180°C for approximately 15 min. All cooking was done in a stainless-steel pot without added salt. After cooking, vegetables were placed on absorbent paper or towels to remove excess water or oil, and inedible portions were discarded as needed. The vegetables were then blended, labeled, stored in polyethylene bags, and frozen at -18°C for later analysis.

**Nitrate and Nitrite Determination**

Nitrite and nitrate levels in vegetables were determined using the Griess reaction after the reduction of nitrates to nitrites. Under acidic conditions, the nitrites react with sulfanilic acid and coupled with N-(1-naphthyl) ethylenediaminedihydrochloride to form a red-violet azo dye. The color intensity was measured via molecular absorption spectroscopy at 540 nm[26].

**Chemicals and Reagents**

All chemical reagents were of analytical grade. Distilled water was used throughout the procedure. Disodium tetraboratedecahydrate (Borax) ($Na_2B_4O_7.10H_2O$, 98% purity) and zinc acetate ($Zn(CH_3COO)_2.2H_2O$, 98% purity) were from VWR chemicals (UK). The ammonia (0.91, 25%) was from Fisher Chemical (UK). Sulfanilamide (98% purity) and Potassium ferrocyanide ($99^+$% purity) were from Acros Organics (India). N-1-naphthyl ethylenediaminedihydrochloride (NED) ($C_{10}H_7NHCH_2NH_2.2HCl$, 98% purity), hydrochloric acid (37%), and Acetic acid glacial (99%) were from Carlo Erba (France). Zinc powder (98%) was from Gainland Chemical Company (GCC, UK), and nitrite and nitrate concentrated standards (1000 mg/L) were from Merck (Germany).

**Aqueous Extraction of Nitrite and Nitrate**

All glassware was thoroughly washed and rinsed with distilled water before use. All chemical materials were weighed using a digital scale (Kern, ABS, d=0.1mg). Aqueous extraction, clarification, and filtrate preparation were performed according to the International Organization for Standardization (ISO) method 6635:1984 [26]. Ten grams of the homogenized sample were weighed into a 100 mL screw-capped bottle. Then, 5 mL of borax solution (0.131 M) and 100 mL of hot distilled water (70–80 °C) were added. The bottles were heated in a water bath for 15 min at 95 °C. Subsequently, 2 mL of Carrez solution I (0.251 M potassium hexacyanoferrate) and 2 mL of Carrez solution II (1.00 M zinc acetate in 3% (v/v) glacial acetic acid) were added. The mixture was diluted in a 200 mL volumetric flask and filtered through CHM filter paper (CHMLAB Group, no. F2041) for subsequent nitrite and nitrate measurements.

**Nitrate Reduction to Nitrite**

Zinc powder was used to reduce nitrate to nitrite[27]. A 10 mL clear filtrate was added to a bottle containing 0.1 g of zinc powder and 5 mL of ammonia buffer (pH 11.0), shaken for 5 min, and then filtered into 50 mL volumetric flasks for the consecutive determination of total nitrate and nitrite.

**Nitrite Determination**

The reduced nitrate ion ($NO_3^-$) and native nitrite ion ($NO_2^-$) were measured by molecular absorption spectroscopy afterGriess reactions [27]. Two mL of Griess reagent I (0.06 M Sulfanilamide in 52.5% (v/v) HCl) were added to a 50 mL volumetric flask containing 10 mL of filtrate and incubated for 5 min. Then, it was combined with 2 mL of Griess reagent II (0.004 M NED) and diluted to volume with distilled water. Absorbance was measured at 540 nm within 15 min using a Shimadzu UV/VIS double-beam spectrophotometer (Japan). Nitrite levels were determined from the nitrite calibration curve for a series of duplicate nitrite standard solutions prepared at eight levels (2.0, 5.0, 10.0, 20.0, 25.0, 30.0, 40.0, and 50.0 mg/L). Linearity expressed by equation and Correlation Coefficient ($r^2$) of the Calibration Curve for Nitrite (NO2-) (Figure 1). Nitrate was calculated as the difference between the total nitrite content after reduction and the initial nitrite. The concentrations are expressed as mg/kg of fresh weight.

**Method Performance**

The method's performance was assessed based on parameters such as the linearity of calibration curves, limit of detection (LOD), limit of quantification (LOQ), accuracy, and precision, in accordance with European Analytical Chemistry guidelines [28].

A calibration curve was created in the range of 2 to 50 µg/mL using duplicate nitrite standard solutions to quantify nitrite in food samples. The limit of detection (LOD)

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was determined as 3 times the response standard deviation for reliable detection beyond a blank sample. The limit of quantification (LOQ), representing the lowest concentration measurable with accuracy, was calculated as 10 times the response standard deviation [28]. The standard deviation was derived from 10 replicate measurements of nitrate and nitrite standards at concentrations of 0.5 and 0.05 mg/L, respectively. The LOD and LOQ were 4 and 14 mg/kg, respectively, for nitrate and 0.13 and 0.45 mg/kg, respectively, for nitrite. The method's accuracy was evaluated using a surrogate recovery experiment in which a known spike was added to a sample and analyzed alongside an unspiked portion. Recovery was determined by comparing the results before and after spiking. Different nitrate levels were tested for two food categories: carrots and cucumbers (low nitrate, <500 mg/kg) with recoveries of 74%–82% and 70%–81%, respectively, and lettuce and beetroot (high nitrate) with recoveries of 94%–98% and 99%–110%, respectively. The recovery rates complied with European Commission standards for nitrate control, with acceptable ranges of 60%–120% for concentrations below 500 mg/kg and 90%–110% for concentrations of 500 mg/kg or higher [29]. No recovery correction was applied to the data.

The method's repeatability (intra-day precision) and intermediate precision (inter-day precision) were evaluated using the relative standard deviation (RSDr) of 6 nitrate analyses across different vegetables. The RSDr for repeatability was 3.5% for cucumber and 6.3% for lettuce, whereas the intermediate precision RSDr ranged from 5.7% to 7.5% for cucumber, carrots, lettuce, and beetroot. Precision was further assessed using Horwitz ratios: the ratio of the observed RSDr calculated from the measured data to the theoretical RSDr calculated as 0.66 times the relative standard deviation under reproducibility conditions (RSDR) predicted from the Horwitz equation ($2^{(1-0.5 \log C)}$), where C is the dimensionless mass fraction of the analyte mean concentration [30]. Intermediate precision Horwitz ratios ranged from 1.1 to 1.65, which is within the acceptable limit (Horwitz ratio < 2) [29].

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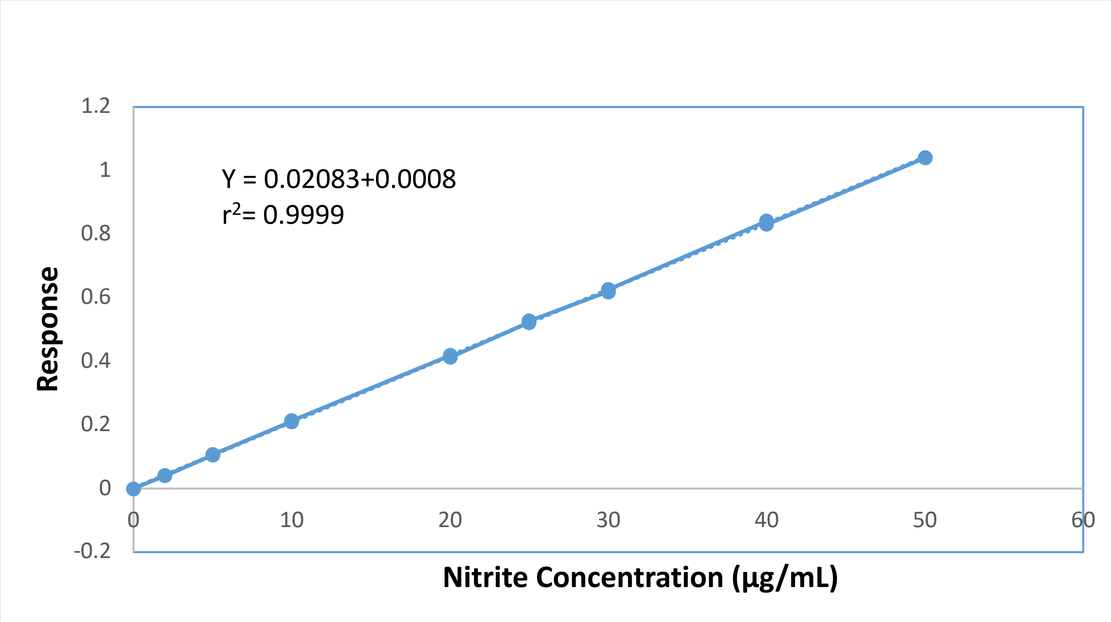


**Figure 1: The calibration curve for Nitrite**

### Statistical Analysis

Statistical analysis was conducted using International Business Machines (IBM) Statistical Product and Service Solutions (SPSS) statistics for Windows, version 22.0 (IBM, Chicago, USA). Descriptive statistics included mean, median, standard deviation, and range. The Shapiro-Wilk test was used to evaluate data normality. Nitrate data within vegetable groups did not follow a normal distribution; therefore, the Kruskal-Wallis test was conducted to compare nitrate levels across various vegetable groups, utilizing Dunn's test and Bonferroni adjustment to manage multiple pairwise comparisons. To assess the effect of cooking on nitrate content between raw and cooked vegetables, the paired mean differences were examined using either a paired Student's t-test or the Wilcoxon signed-rank test, depending on the normality of the differences. $p< 0.05$ as a significance threshold was applied to all tests.

## RESULTS AND DISCUSSION

### Nitrate Levels in Raw Vegetables and Vegetable-Based Dishes

Table 1 presents the results of the spectrophotometric determination of nitrate and nitrite from 270 samples representing 36 different types of vegetables. Vegetables were stratified based on the edible part of the plant [31] and compared with a newly created database of nitrate content in plant-based foods [12] (Table 1). Nitrates were detected in 94.7% of the samples, with average concentrations ranging from 6.13 mg/kg in sweet corn to 1721.22 mg/kg in celery. Figure 2 shows average nitrate levels in different types of vegetables, where error bars indicate standard errors. Figure 3 demonstrates average nitrate levels in vegetable groups categorized by

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edible parts. The levels of nitrate in vegetable groups were arranged in the following descending order: Leafy and stem vegetables (467.93 mg/kg) > root and tuber vegetables (394.14 mg/kg) > fruit vegetables (142.48 mg/kg) > legume and seed vegetables (106.54 mg/kg) > flower vegetables (63.32 mg/kg) > bulb vegetables (30.36 mg/kg) (Figure 3). This arrangement corresponds to earlier trends in nitrate concentrations among vegetable groups[32].

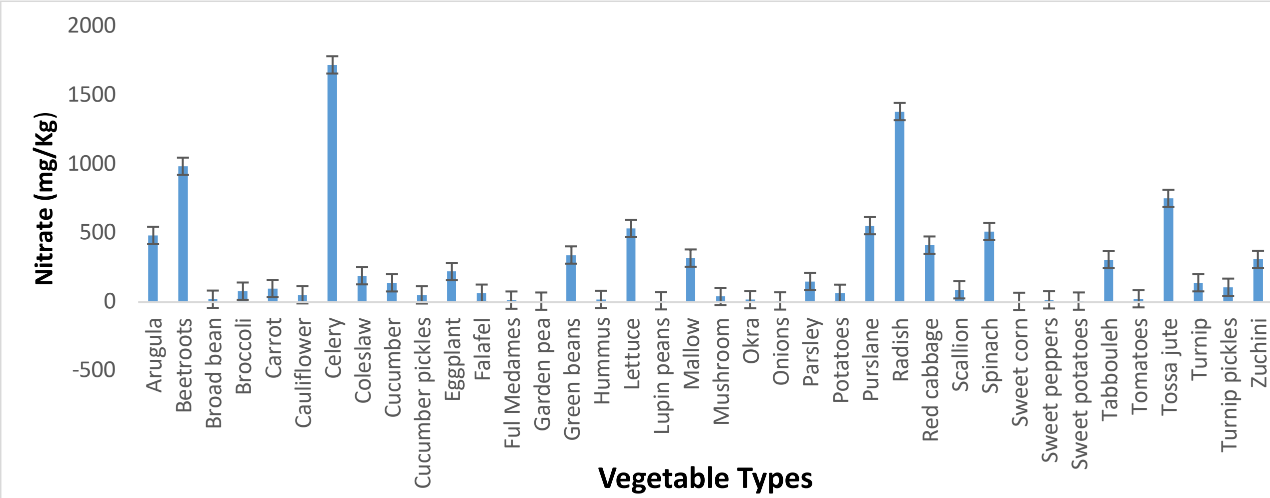


**Figure 1: Mean Nitrate Levels (mg/kg) in Vegetable Types**

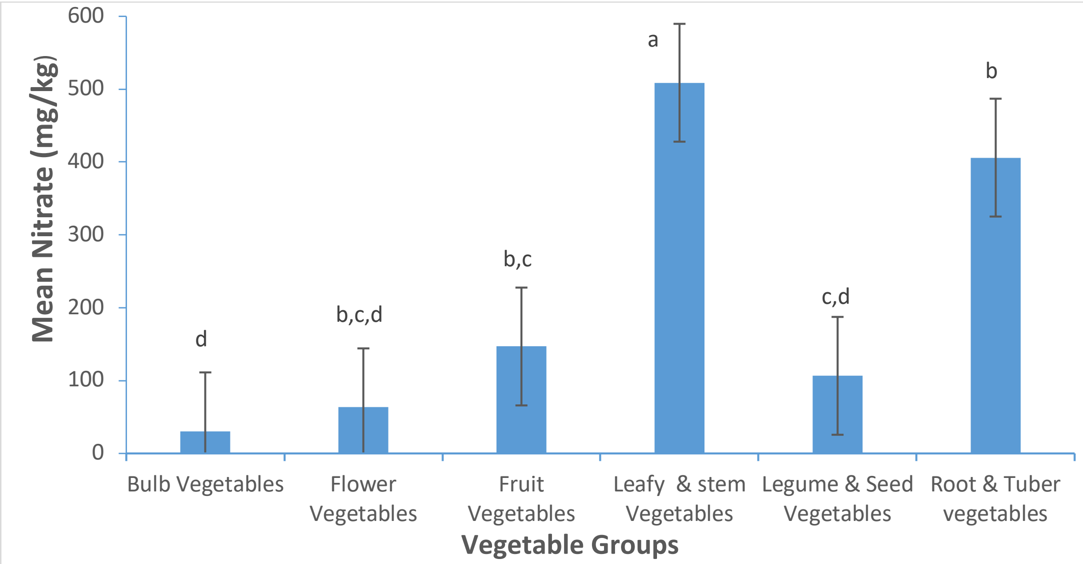


**Figure 2: Mean Nitrate levels (mg/kg) in Vegetable Groups**

Nitrate levels in vegetables were categorized as very low (<20 mg/kg), low (<200 mg/kg), medium (200–1000 mg/kg), high (1000–2000 mg/kg), and very high (>2000 mg/kg) [12]. Of the vegetables examined, 21.1% exhibited very low nitrate levels, 42.1% low, 31.6% medium, and 5.3% high. Among the leafy and stem vegetables, celery exhibited the highest nitrate levels, with an average of 1721.2 mg/kg and a maximum of 2246 mg/kg. Conversely, other green leafy vegetables like Tossa jute (Molokhia in Arabic), purslane, lettuce, spinach, mallow, and arugula contained moderate nitrate concentrations, which were lower than those of comparable vegetables in the reference database for plant foods [12]. Tabbouleh salad also had a moderate nitrate content, with an average of 309.14 mg/kg and a peak of 736.1 mg/kg, exceeding the nitrate levels found in parsley (average 150.83 mg/kg, max 389.32 mg/kg). Ingredients in tabbouleh, such as tomatoes, mint, and raw bulgur, likely contributed to its nitrate content. By comparison, coleslaw had a lower nitrate level, with an average of 191.57 mg/kg and a maximum of 353.42 mg/kg. Among fruit vegetables, zucchini exhibited the highest average nitrate content at 313.96 mg/kg, followed by eggplant at 221.56 mg/kg and cucumbers at 130.5 mg/kg. Tomatoes, bell peppers, okra, and flower vegetables, showed lower nitrate levels, averaging below 100 mg/kg.

Root and tuber vegetables were divided into two categories according to nitrate content. Radishes and beetroots exhibited high nitrate levels with average levels of 1382.4 and 985.9 mg/kg, respectively, with maximum concentrations exceeding 1500 mg/kg. Conversely, the second group—consisting of carrots, potatoes, sweet potatoes, turnips, and turnip pickles—exhibited low nitrate levels, with an average of less than 200 mg/kg. Conversely, legumes and seed pods, including hummus, falafel, and fulmedames, had very low nitrate levels, except for green beans, which demonstrated a relatively higher mean nitrate content of 342.02 mg/kg, with a maximum exceeding 500 mg/kg.

According to this study, nitrate levels in Jordanian vegetables are generally comparable to or lower than those in other regions, with considerable variability within each vegetable type, as indicated by the high standard deviations observed (Table 1). The variability in nitrate levels among vegetable types was observed, likely due to factors such as plant genotype, cultivar, age, light intensity, humidity, temperature, fertilizer use [13, 32], harvest timing, and cultivation methods [22, 23]. Jordan's high light intensity, with over 300 sunny days per year[33], likely contributed to the lower nitrate content observed, as increased light activates nitrate reductase activity, converting nitrate to ammonium [32].

In terms of nutrition, vegetables are classified by their nutrient profile through food pattern modeling[34], such as USDA's dietary patterns ' USDA's MyPlate '[3], into categories like dark green, red and orange, beans, peas and lentils, starchy and others.

According to the present study, dark green vegetables had the highest nitrate content (403.7 mg/kg ± 323.55, $p < 0.05$), followed by other vegetables (344.06

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mg/kg ± 482.27 mg/kg) (Table 2). The wide variation in nitrate content among vegetable types may help individuals control nitrate intake when adhering to healthy eating patterns, such as vegetable-rich diets. As an illustration, two Dietary Approaches to Stop Hypertension diet models (DASH diet) with high and low nitrate levels (1222 mg/kg vs. 174 mg/kg) demonstrate that greater vegetable consumption does not necessarily lead to higher nitrate intake[5].

**Influence of Cooking on Nitrate Content**

Subsamples of selectedfresh vegetables (n= 91) were cooked by boiling, steaming, deep-frying, stir-frying, or baking, and their nitrate levels were measured before and after. Table 3 presents the mean and standard deviation of nitrate concentrations in raw and cooked vegetables and the average ratios of nitrate gain and loss after cooking. Figure 4 compares the overall effects of different cooking methods on vegetable nitrate content. The chart compares nitrate concentration (mg/kg) in raw and cooked vegetables, with each cluster representing a different cooking method. Error bars represent standard errors. Bars within each cluster that share the same number of asterisks are not significantly different from each other *(p*> 0.05), based on the paired t-test for all methods except boiling, which used the Wilcoxon signed-rank test.

Boiling reduced the nitrate levels across vegetable types significantly ($p<0.05$) as well as among the individual boiled vegetables ($p < 0.05$), except for carrots, which showed no statistically significant difference in nitrate content compared to raw counterpart ($p>0.05$) (Table 3). However, the ratio of reduction varied by vegetable type: Potatoes and green beans had the lowest (22%) and highest (40%) average reduction ratios, respectively (Table 3). The reduction in nitrate content after boiling could be related to the high solubility of nitrate in water[6]and the prolonged heat treatment that softens vegetable tissue, causing water absorption, nitrate leaching, and weight gain [17, 35].

According to previous studies, the ratios of nitrate reduction following boiling were for potatoes between 23.3% and 59.7% [16-19, 36]; for beetroots and green beans, these were 10% and 15%, respectively [19]; and for carrots, they ranged from 13% to 74% [36].The extent of nitrate reduction is primarily affected by the strength of the cell walls; firmer vegetables impede heat penetration and reduce nitrate loss [37]. It can also be affected by how the vegetables are prepared before cooking, especially if peeled, because the peel protects against nitrate loss [16, 21]and the type of water used, like tap water instead of distilled water, which can contain nitrate [38].

The influence of steaming on nitrate levels varies among vegetable types, resulting in increases in cauliflower (76.5%), decreases in carrots (34.0%), and negligible effects in zucchini (3.63%). Variations in vegetable responses to steaming are likely

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due to differences in cell structure, surface area, water content, and nitrate distribution among vegetable types. Overall, steaming did not produce a statistically significant effect across vegetable types ($p > 0.05$) .This result is consistent with those of earlier studies [16, 19, 20], although other research indicated reductions in nitrate concentrations in steamed potatoes (16%)[21]and cabbage (33%) [20].

Deep-frying significantly increased nitrate content in potatoes and eggplants by 1.7 and 1.1 times, respectively ($p < 0.05$), but did not result in a statistically significant change in cauliflower, despite a 4.1-fold increase (Table 3). Previous research has shown that deep-frying vegetables raise nitrate content by 1.6 to 3.3 times for leafy vegetables [35], 52% to 160% for potatoes[16, 17, 19], 2 to 3 times for root vegetables [36], and 12.4% to 29.9% for other fried vegetables [15]. However, some studies have reported reductions in nitrate levels in fried potatoes, with decreases of 32%–60% [18, 21].

The stir-frying increased the nitrate content of vegetables ($p < 0.05$). However, the increase in nitrate content was significant only for stir-fried mallow, with a 1.8-fold increase ($p < 0.05$), but not for stir-fried spinach or beans, despite increases of 1.7 and 1.5 times, respectively ($p > 0.05$). Similarly, Wu *et al.* [20] reported a 31% increase in nitrate levels in stir-fried water spinach [20], whereas no significant change was observed in stir-fried cabbage [19, 20].

Nitrate levels in baked eggplant and potatoes were significantly higher than in their raw form, with increases of 1.7 and 1.3 times, respectively ($p > 0.05$). Zucchini exhibited a 1.8-fold increase in nitrate content; however, this result was not statistically significant ($p > 0.05$). Prasad and Chetty [36] reported that nitrate levels in leafy vegetables like cabbage, celery, and lettuce remain stable after baking. In other research, the nitrate content of baked root vegetables, including potatoes, sweet potatoes, and carrots, increased slightly, with a range of 2.80%–8.43% [36].

The increases in nitrate content observed after deep-frying, stir-frying, and baking are likely due to the concentration effects of these dry cooking methods, which result from water evaporation. However, given the water solubility of nitrates [6], and limited data on the presence of nitrate in oils, the possibility of nitrate contamination from oils cannot be entirely excluded, as oils may contain nitrogenous compounds such as ammonia [17].

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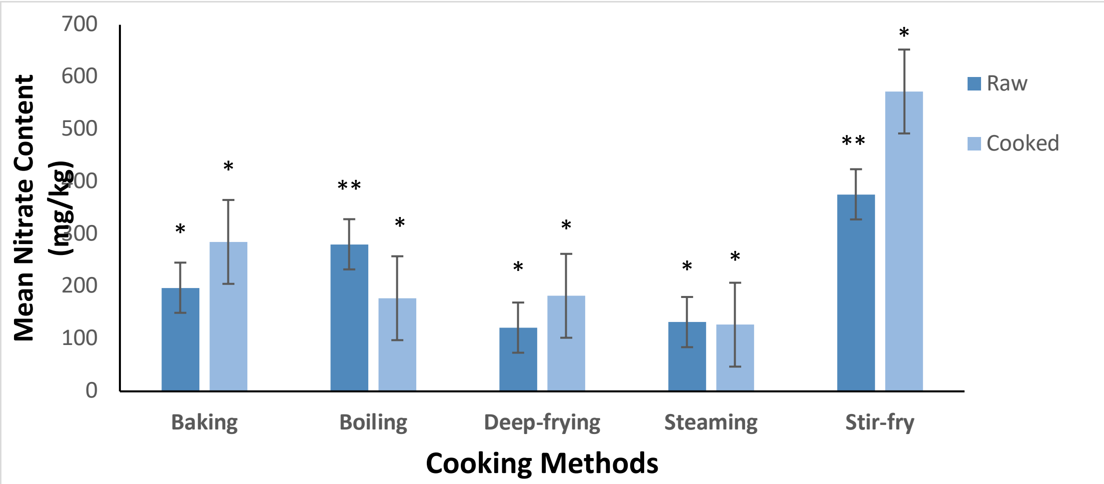


**Figure 3: Mean Nitrate Content (mg/kg) in Raw and Cooked Vegetables**

### Nitrite in Raw and Cooked Vegetables

Vegetable samples exhibited low nitrite content, averaging below 1 mg/kg, with 76.7% of the 361 samples showing undetectable levels. These results align with findings from other studies [32, 39].

This study has limitations that may affect the generalizability and accuracy of the findings, including sampling from a single region, restriction to winter months, and lack of control over agricultural practices. In addition, due to limited resources, only a single-replicate cooking trial was conducted, which limited the assessment of variations in the cooking process. However, reliable measures could be relatively ensured due to the thorough in-house validation and repeatability measures.

## CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

This study provides essential baseline data on nitrate levels in vegetables commonly consumed in Jordan, emphasizing the impact of different cooking methods. Nitrate levels in Jordanian vegetables are comparable to or lower than those in other countries. Leafy and stem vegetables have the highest nitrate contents, followed by root vegetables. Boiling reduced nitrate content in some vegetables, whereas deep-frying, stir-frying, and baking increased it, steaming had no effect. These findings are critical for public health and food safety agencies when assessing dietary nitrate exposure and developing evidence-based recommendations for safe nitrate intake. Future research should explore geographical and seasonal variations and additional cooking methods to enhance our understanding of nitrate exposure.

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Administration (JFDA) for technical support in laboratory facilities and research equipment.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

**Table 1: Nitrate Content (mg/kg) for Raw Vegetables and Vegetable-Based Dishes commonly consumed in Jordan Compared with Reference Database[1]**

| Vegetable type | Nitrate content in the present study (mg/kg) | | | | | Nitrate content in reference database [1] (mg/kg) | | |
|---|---|---|---|---|---|---|---|---|
| | (N)[2] | Range | Median | Mean* | SD | Median | Mean | SD |
| **Leafy and stem vegetables** | **86** | **12.5 - 2246.0** | **363.89** | **508.85** [a] | **456.48** | | | |
| Arugula *(Erucavesicaria sativa)* | 7 | 87.0 - 862.3 | 548.1 | 484.8 | 305.6 | 4506.1 | 4202.2 | 1997.2 |
| Mallow *(Malvaspp)* | 8 | 72.6 - 742.4 | 243.3 | 319.7 | 251.2 | N. F | N. F | N. F |
| Spinach (*Spinaciaoleracea)* | 15 | 111.1 - 1489.8 | 492.8 | 512.6 | 349.3 | 1757 | 1807.5 | 1105.2 |
| Tossa jute *(Corchorusolitorius)* | 7 | 230.7 - 1324.2 | 851.3 | 753.1 | 461.1 | N. F | N. F | N. F |
| Lettuce *(Lactuca sativa)* | 13 | 186.7 - 1183.1 | 485.1 | 534.9 | 302.0 | 1078 | 1167.2 | 666.0 |
| Parsley *(Petroselinum crispum)* | 7 | 12.5 - 389.3 | 98.3 | 150.8 | 142.6 | 735 | 1017.7 | 971.0 |
| Purslane *(Portulacaoleracea)* | 5 | 224.1 - 943.5 | 511.2 | 554.7 | 328 | 3001.7 | 3059.1 | 1577.8 |
| Red cabbage (*Brassica oleracea*) | 5 | 153.9 - 1007.1 | 318.4 | 413.9 | 342.5 | 337.7 | 364 | 266.4 |
| Celery *(Apiumgraveolens)* | 5 | 982.3 - 2246.0 | 1650.1 | 1721.2 | 535.3 | 1251.8 | 1646.2 | 1353.3 |
| Coleslaw salad | 8 | 96.3 - 353.4 | 163.3 | 191.5 | 86.9 | N. F | N. F | N. F |
| Tabbouleh salad[3] | 6 | 98.0 - 736.0 | 181.2 | 309.1 | 263 | N. F | N. F | N. F |
| **Fruit vegetables** | **49** | **ND - 542.1** | **117.10** | **146.80** [b, c] | **138.32** | | | |
| Cucumber *(Cucumissativus)* | 9 | 10.9 - 218.3 | 158.0 | 140.2 | 73.2 | 180.7 | 247.3 | 238.1 |
| Cucumber pickles | 6 | ND - 99.7 | 57.4 | 52.5 | 35.6 | N. F | N. F | N. F |
| Tomatoes *(Solanumlycopersicum)* | 7 | ND-117.1 | 3.8 | 24.5 | 43.8 | 58.5 | 237.4 | 618.6 |
| Eggplant (Solanummelongena) | 10 | 69.2 - 353.6 | 203.7 | 221.5 | 97.3 | 289.2 | 348.9 | 263.4 |

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| Vegetable type | Nitrate content in the present study (mg/kg) | | | | | Nitrate content in reference database [1] (mg/kg) | | |
|---|---|---|---|---|---|---|---|---|
| | (N)[2] | Range | Median | Mean* | SD | Median | Mean | SD |
| Bell peppers *(Capsicum annuum)* | 3 | ND - 33.8 | 14.6 | 16.8 | 16.0 | 75.8 | 662.6 | 1634.9 |
| Zucchini *(Cucurbita pepo cylindrica)* | 10 | 100.2 - 542.1 | 331.1 | 310.6 | 138.1 | 494.0 | 533.1 | 447.9 |
| Okra *(Abelmoschusesculentus)* | 4 | 8.73 - 29.0 | 17.0 | 17.9 | 8.38 | 70.0 | 112.5 | 102.1 |
| | | | | | | | | |
| **Flower vegetables** | **22** | **ND - 333.4** | **48.93** | **63.32[b, c,d]** | **66.97** | | | |
| Mushroom *(Agaricusbisporus)* | 4 | 23.0 - 52.7 | 45.5 | 41.7 | 12.9 | 15.0 | 44.8 | 100.6 |
| Broccoli *(Brassica oleracea)* | 10 | ND - 333.4 | 41.2 | 80.0 | 97.7 | 209.0 | 370.4 | 728.6 |
| Cauliflower *(Brassica oleracea)* | 8 | 22.4 - 78.0 | 53.7 | 53.2 | 18.0 | 140.0 | 243.1 | 343.1 |
| | | | | | | | | |
| **Root and tuber vegetables** | **46** | **ND - 2401.9** | **90.91** | **406.07[b]** | **602.75** | | | |
| Beetroots *(Beta vulgaris)* | 8 | 514.5 - 1767.4 | 807.8 | 985.9 | 509.4 | 1384.5 | 1541.1 | 1196.4 |
| Radish *(Raphanussativus)* | 6 | 673.2 - 2401.9 | 1118.4 | 1382.3 | 701.8 | 1370.0 | 1503.7 | 1169.7 |
| Carrot *(Daucuscarota)* | 14 | ND - 285.7 | 55.7 | 99.4 | 103.2 | 100.0 | 159.4 | 170.1 |
| Sweet potatoes *(Ipomoea batatas)* | 6 | ND - 15.9 | 7.64 | 7.8 | 5.72 | 32.0 | 87.5 | 184.8 |
| Potatoes *(Solanumtuberosum)* | 7 | 25.3 - 92.5 | 71.5 | 64.5 | 24.3 | 144.0 | 159.4 | 110.9 |
| Turnip *(Brassica rapa)* | 2 | 120.6 - 160.6 | 140.6 | 140.66 | 28.3 | 245.0 | 874.1 | 1835.5 |
| Turnip pickles (Brassica rapa) | 3 | 16.7 - 235.2 | 73.1 | 108.4 | 113.4 | N. F | N. F | N. F |
| | | | | | | | | |
| **Legume and seed vegetables** | **52** | **ND - 670.4** | **19.35** | **106.54[c, d]** | **173.28** | | | |
| Sweet corn (*Zea mays L.)* | 4 | ND - 11.1 | 5.62 | 6.13 | 3.72 | 21.4 | 194.5 | 374.3 |
| Lupinpeans (*Lupinusalbus* L.) | 3 | 4.65 - 13.5 | 11.5 | 9.91 | 4.66 | N. F | N. F | N. F |

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| Vegetable type | Nitrate content in the present study (mg/kg) | | | | | Nitrate content in reference database [1] (mg/kg) | | |
|---|---|---|---|---|---|---|---|---|
| | (N)[2] | Range | Median | Mean* | SD | Median | Mean | SD |
| Green beans *(Phaseolus vulgaris)* | 14 | 159.3 - 670.4 | 315.2 | 342.0 | 185.7 | 310.5 | 334.6 | 167.3 |
| Garden pea *(Pisumsativum)* | 8 | ND - 17.1 | 7.92 | 7.57 | 6.53 | 30.0 | 201.7 | 338.6 |
| Broad bean *(Viciafaba)* | 9 | ND - 44.5 | 9.7 | 21.6 | 19.1 | N. F | N. F | N. F |
| Hummus[4] | 6 | ND - 44.0 | 18.1 | 20.3 | 16.0 | N. F | N. F | N. F |
| Falafel[5] | 4 | 39.0 - 118.4 | 51.6 | 65.1 | 36.9 | N. F | N. F | N. F |
| Ful Medames[6] | 4 | ND - 41.9 | 8.95 | 14.9 | 18.8 | N. F | N. F | N. F |
| **Bulb vegetables** | **15** | **ND - 182.6** | **7.51** | **30.36$^{d}$** | **57.46** | | | |
| Onions *(Allium cepa)* | 11 | ND - 32.4 | ND | 8.82 | 12.5 | 86.5 | 218.4 | 547.2 |
| Scallion *(Allium cepa)* | 4 | ND - 182.6 | 86.3 | 89.6 | 92.2 | 318.6 | 371.6 | 297.7 |

Note. N=270; ND: non-detected (<limit of detection (LOD)); N.F: not found
*Means values in column with different subscripts differ significantly ($p < 0.05$) (pairwise comparisons using the Dunn-Bonferroni test)
[1]Zhong *et al*. [12]
[2] Each sample was analyzed in a single replicate
[3] Salad made of finely chopped parsley, with tomatoes, mint, onion, soaked uncooked bulgur
[4] Dip made from boiled chickpeas blended with tahini
[5] Deep-fried patty made from a mixture of ground chickpeas and fava beans
[6] Stewed fava beans flavored with lemon juice and garlic

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**Table 2: Nitrate Content (mg/kg) Based on USDA's MyPlate Classification for Vegetables**

| Vegetable Group [a] | N | Min | Max | Mean* | SD |
|---|---|---|---|---|---|
| Dark green vegetables | 53 | ND | 1489.8 | 403.7 [a] | 323.5 |
| Red and orange vegetables | 30 | ND | 285.7 | 55.3 [b] | 83.6 |
| Beans, peas and lentils | 14 | ND | 118.4 | 31.6 [b] | 31.4 |
| Starchy vegetables | 19 | ND | 92.5 | 28.2 [b] | 32.0 |
| Other vegetables | 154 | ND | 2401.9 | 344.0 [a] | 482.2 |

*Note.* ND: non-detected (<limit of detection (LOD))

[a]Based on the USDA's MyPlate classification, vegetables were categorized according to their nutrient profiles [3]

*Means value in the column with different subscripts differ significantly ($p < 0.05$) (pairwise comparisons using the Dunn-Bonferroni test)

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**Table 3: Nitrate Content (mg/kg) in Selected Vegetables Before and After Cooking**

| Cooking Methods [a] | (n)[b] | Raw | | Cooked | | Loss (-) Gain (+) | *P* |
|---|---|---|---|---|---|---|---|
| | | Mean (mg/kg) | SD | Mean (mg/kg) | SD | | |
| **Boiling** | | | | | | | |
| Broccoli | 6 | 118.6 | 111.5 | 67.8 | 44.6 | -32.7 | 0.014* |
| Cauliflower | 8 | 53.2 | 18.0 | 32.2 | 18.8 | -35.2 | 0.016* |
| Carrots | 4 | 182.3 | 106.0 | 135.4 | 130.8 | -35.1 | 0.068** |
| Beetroot | 7 | 961.1 | 545.3 | 593.8 | 279.7 | -34.7 | 0.027* |
| Potatoes | 6 | 71.0 | 18.7 | 55.4 | 20.4 | -22.0 | 0.028** |
| Green beans | 6 | 228.2 | 109.4 | 147.5 | 125.1 | -40.6 | 0.002* |
| **Steaming** | | | | | | | |
| Cauliflower | 4 | 46.6 | 24.2 | 84.8 | 62.1 | +76.57 | 0.068** |
| Carrots | 4 | 127.1 | 98.3 | 78.2 | 69.7 | -34.09 | 0.137* |
| Zucchini | 4 | 325.5 | 184.9 | 294.6 | 105.0 | +3.63 | 0.584* |
| **Deep-frying** | | | | | | | |
| Cauliflower | 4 | 46.6 | 24.2 | 168.2 | 119.7 | +311.5 | 0.108* |
| Potatoes | 5 | 71.0 | 20.9 | 115.2 | 39.7 | +65.4 | 0.031* |
| Eggplant | 6 | 213.9 | 80.1 | 247.9 | 108.3 | +13.5 | 0.003* |
| **Baking** | | | | | | | |
| Potatoes | 5 | 71.0 | 20.9 | 93.8 | 32.3 | +31.0 | 0.023* |
| Zucchini | 5 | 303.0 | 179.9 | 428.9 | 225.1 | +74.7 | 0.109* |
| Eggplant | 4 | 225.1 | 97.5 | 345.3 | 174.7 | +67.1 | 0.037* |
| **Stir-frying** | | | | | | | |
| Green beans | 4 | 251.6 | 132.3 | 401.6 | 291.3 | +49.0 | 0.158* |
| Spinach | 5 | 366.6 | 300.0 | 497.3 | 335.9 | +72.8 | 0.103* |
| Mallow | 4 | 512.9 | 206.5 | 837.7 | 165.9 | +78.2 | 0.012* |

[a] Specific cooking methods were chosen for each vegetable based on traditional Middle Eastern practices [24, 25]
[b] Each sample was cooked once and analyzed in duplicate
SD: standard deviation
*p < .05 in Student's t-test.; **p < .05 in Wilcoxon signed ranks test for paired data

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