Reduction Nitrate Content from Contaminated Vegetable, by Hard Shell of Wild Endemic Almonds: *Amygdalus lycioides* and *Amygdalus wendelboi*

Parisa Ziarati, Bernhard Hochwimmer

**Abstract**

The aim of current study was to introduce a new and economic method for reduction of nitrate contents in mostly consumed leafy vegetables. The nitrate content was determined before/after treatments by powder of modified shell of two wild endemic almonds using spectroscopic method. Adsorption capacity of nitrate onto modified almond hard shells by Phosphoric acid of *Amygdalus lycioides* and *Amygdalus wendelboi* collected from south of Iran was investigated by considering the effects of various parameters like contact time, initial concentrations, pH, absorbent dose and particle size. The effect of treatment time at three different time point (10, 15 and 20 minutes of soaking) was determined. The results revealed that nitrate and nitrite contents of celery, Iceberg and Romani lettuce and carrot were decreased significantly \((p<0.05)\). Different concentration of almonds’ shell did not influence sensory attributes of vegetables. The most efficient time for treating by powder of shells was 15 min, but the most practical one is recommended 20 min. Addition of almonds’ shell did not change pH of all studied vegetable samples. Application of modified shell of wild almond suggested as a novel, safe and economic method for removal of nitrate in contaminated crops and vegetables.

**Keywords**

Wild Almond; Contaminated Vegetable; Adsorbent; Nitrate Content

**Introduction**

Nitrate content is an important quality characteristic of vegetables. Vegetable nitrate content is of interest to governments and regulators owing to the possible implications for health and to check that controls on the content are effective. Nitrate itself is relatively non-toxic but its metabolites may produce a number of health effects [1-5]. The vast industrial waste materials and sewages from a lot factories and different chemical fertilizers and pesticides in Tehran have caused contamination of soils [6, 7]. The soil and water of other cities especially in the north of Iran has been already contaminated. According to the last studies, Gilan Province, owing to its rich natural resources, is one of the most populated provinces in Iran [8, 9]. Irrigated agriculture is one of the most well-known causes of groundwater contamination throughout the Gilan state (south Caspian Area) in the north of Iran. According to the provincial statistics in 1998, Rasht City 1.4 million m³ untreated sewage into the river which is attributed to the increasing urban development [7, 10]. Nitrates and...
nitrites seemed to be among the chemicals that may cause pollution; many studies have expected the effect of these compounds on the environment and on the living health. These studies focused on the nitrate and nitrite contents in water sources and in vegetables consumed by humans.

In order to control the nitrate and nitrite intake by consumers in general and on babies in particular who are the most vulnerable to the advance effects of these two compounds, a maximum acceptable limit of these compounds were suggested. The Acceptable Daily Intake (ADI) of nitrate and nitrite set by European Commission’s Scientific Committee for Food (ECSCF), is 3.7 mg/kg body weight, and 0.06 mg/kg body weight, respectively [3-7]

The USA Environmental Protection Agency (EPA) Reference Dose (RfD) for nitrate is 1.6 mg nitrate nitrogen kg\(^{-1}\) bodyweight (bw) per day (equivalent to about 7.0 mg NO\(_3\) kg\(^{-1}\) bw per day) [3-5]. The JECFA and SCF have proposed an ADI for NO\(_3\), of 0-0.078,9 and 0-0.06 mg NO\(_3\) kg\(^{-1}\) bw 32, respectively, while the EPA has set an RfD of 0.1 mg nitrite nitrogen kg\(^{-1}\) bw per day (equivalent to 0.33 mg NO\(_2\) kg\(^{-1}\) bw per day). 5 The SCF retains that the ADIs are applicable to all sources of dietary exposure. [3-5]

For methemoglobinemia in infants, it was confirmed that the existing guideline value for nitrate ion in drinking water of 50 mg/L is protective. For nitrite, human data reviewed by (JECFA: The Joint FAO/WHO Committee on Food Additives) support the current provisional guideline value of 3 mg/L [6-11]. Agriculture is considered the major source of nitrate and nitrite in the environment. [10]. In current study (in the South of Tehran), farmers use higher amounts of fertilizers than they should, either due to their ignorance, or because they want to increase their production quickly when the prices of their products in markets are high. Plants cannot utilize all the added fertilizers, so the excessive amounts will be widespread by irrigation water through the soil to reach ground water, or it may dissolve in runoff water and flows into streams or takes and rivers. Nitrogen compounds are also accumulate in some plant issues [8, 9].

More than three quarters of our average nitrate intake comes from vegetables [8], which provide about 80% of the average daily dietary intake [7-9]. Vegetables that may accumulate nitrate in their tissues are leafy vegetables such as Spinach, Lettuce, and Cabbage, or root crops like carrot, Potatoes, and others like Cauliflower, beans and peas. The amount of nitrate in vegetables like lettuce is usually ranges among 90 and 3520 [7-10]. The excessive use of the pesticides and fertilizers in agriculture with the threat of these chemicals in crops and water has become one of the most important public awareness issues. The correlation between consuming food with high levels and the formation of carcinogenic nitrosamines compounds has been studied. Permanent contaminants such as metals or nitrate may be transferred to higher levels in the food chain through environmental expansion. The levels of these contaminants, due to environmental socialization of species are generally much higher in marine physico than the surroundings [11-15]. Using a long-term freezing process is a good way to reduce nitrate and nitrite in vegetables. Given the significant daily consumption of vegetables by the people and its positive impact on community health, consideration of other maintenance processes is important [Almond (Rosaceae family) with wide applications in pharmaceutical, oleo chemicals, food and cosmetic industries is considered as a pleasant nut throughout the world. Other than the regular almond, there are more than thirty wild or partially cultivated almond species in the world of which twenty species have been reported in Iran [16, 17]. Amygdalus wendelboi is an endemic species distributed just in south of Iran, in Mountains of Gnow protected area [16]. The fruits locally called “Archen”, has a wide application in folk medicine to treat cold, cough, headache and skin burns. Amygdalus lycioides locally called “Badamak” is one of the endemic species found in some parts of Iran especially in south regions [16, 17]. It is traditionally used as antidiabet, antinflammation, antibacterial and laxative agent [6]. After removing the bitterness from the kernel oil, it could be used as edible nutritive oil. Several studies have been reported on A. lycioides different parts. Phenolic and flavonoid contents of this plant determined as 233 and 13.4 mg/g [16, 17]. These are locally used as edible kernels by people. In this study the potential of two wild endemic almond: Amygdalus lycioides and Amygdalus wendelboi shells was assessed for adsorption of nitrate from mostly consumed vegetable after rinsing and during soaking in different times. On the whole, the objectives of the present work are:

- Determination of the level of nitrate and nitrite in Lettuce, Celery, Spinach, Potato, Cabbage, Chinese cabbage, Eggplant and Cauliflower sold in Tehran markets.
- Determination the effect of adding modified Shell of

Wild Endemic Almonds:  
*Amygdalus lycioides* and *Amygdalus wendelboi* as adsorbent after washing & rinsing by water and during soaking vegetables in different times on the reduction level of nitrate and nitrite content in leafy vegetables.

Materials and Methods

Study of Area

a) Adsorbent Collection

*A. lycioides* and *A. wendelboi* fruits and their shells were collected in June 2013 from Sirmand Mountains near Hadji-Abad County, Hormozgan Province and the mountains in Geno protected area, Bandar-Abbas, Hormozgan Province respectively. Both samples were identified by R. Asadpour. The Geno Biosphere Reserve, with a total area of 27,500 hectares, situated in the Hormozgan province of Iran. It has been designated as a protected area by the Iranian Department of Environment in 1976. The area is mountainous region that located among plains and hills. The region’s geographical location “18 ° 27 to” 29 ° 27 north latitude and “18 ° 56 to” 56 ° 55 ‘east longitude, is located in the north of Bandar Abbas (Figure 1). Plain part of the region includes much of the southern, eastern and northern part of the strip consisted of alkaline and saline soils contain large amounts of soluble salts such as chloride, sulfate and carbonate of Ca, Mg, sodium, and potassium [18,19].

![Location of *Amygdalus lycioides* and *Amygdalus wendelboi* Sampling Collection](image)

Shell samples were washed several times with deionized water and dried (sun or mechanical). The adsorbents were then ground in a blender and stored for further use. All reagents and chemicals were used of analytical reagent grade and were obtained from standard sources.

Phosphoric acid, Activated carbon, Potassium dichromate and other chemicals were obtained from standard sources.

b) Vegetables Collections Area

Root and leafy vegetables were collected for this study: Iceberg and Romania Lettuce, Celery, Potato, Chinese cabbage, Carrot, Celery and Cabbage. These are the most common purchased vegetables in Tehran markets; in addition most of them are usually consumed fresh, and grown above the ground.

A systematic survey of nitrate and nitrite in vegetables and crops from 860 samples (from 10 different markets during 12 consecutive months of 2015) in Tehran was carried out.

Adsorption capacity of nitrate onto modified almond shells by Phosphoric acid was investigated by considering the effects of various parameters like contact time, initial concentrations, pH, absorbent dose and particle size.

Nitrate and Nitrite Extraction

A fifty gram sample of the prepared vegetable and crop samples was blended with 50 mL distilled water in a home blender. The mixture was filtered through Whatman No.2 filter paper, and the filtrate was passed through a glass column fitted with a tape and filled with activated alumina, in order to separate the green color (Chlorophyll)
and get a transparent solution. Water was used as eluting solvent. The eluted solution filtered using 0.45 μm filter paper in order to eliminate the turbidity and get a clear solution.

Quantitative Determination

a) Quantitative Determination of Nitrate

10 mL of the transparent, clear solution was analyzed for the nitrate content using a HANNA Instrument “which gives the nitrate content of sample as NO₃⁻⁻N.

b) Quantitative Determination of Nitrite

With the AOAC official Methods 973/31 [20]. A portion of solution containing nitrite was transferred into a 25 mL volumetric flask. Then 2.5 mL sulfonamide were added, followed by addition of 2.5 mL NAD [N−91−naphthyl]ethylenediamin.2HCl]. The volume was complete with water and left 15 minutes in order to give time for color development. The absorbance was measured at 545 nm against a blank solution. The nitrite concentration was determined using the calibration curve solutions of 0.2, 0.4, 0.6, and 0.8 ppm NaNO₂. The absorbance values were measured at 545 nm. The calibration curve was constructed by plotting the absorbance vs. the concentration.

Statistical Method

One-parametric Kruskal–Wallis/Mann–Whitney U tests were applied to compare differences between objects. Non-parametric multiple comparison test (Dunn’s test) was performed to determine statistical significance of results at α = 0.05. The GLM procedure was used for analysis of different time, pH and particle size adsorbent treatments with means separated by Duncan’s multiple range test at p<0.05. The CORR procedure was used for correlation analysis with means separated at p<0.05.

Results

The intakes of nitrate and nitrite from food were calculated as a global level on the basis of mean food consumption in the GEMS/Food regional diet (WHO1998) [6, 21], and the mean concentrations in food in Europe from the submitted data can be seen in tables 1 and 2. Intake from drinking water was added, assuming a water consumption of 2 L/day. The mean concentration in water that was used in the intake calculation was 4 mg/L for nitrate and 0.3 mg/L for nitrite which was representative of the usual concentrations found in water [22]. An average body weight of 60kg was used for the global intake assessment [22].

The nitrate and nitrite content in several crops collected from 10 different markets of Tehran were analyzed to determine if the location has an effect on the nitrate and nitrite concentration in crops. The results show no significant effect of the region on the nitrate and nitrite content in different leafy vegetable crops.

Nitrate and nitrite content in leafy, root and vegetable samples were determined in Tehran markets as showed in Table 1 for nitrate, and in Table 2 for nitrite before and after treating by modified shell of wild almonds after 15 minutes of soaking in water and being vicinity of studied adsorbents. The adsorption was pH solution dependent and the maximum adsorption was observed at solution pH of 4.8.

Table 1: The Mean Content of Nitrate (NO₃⁻⁻) Content mg/kg FW in the Vegetables Surveyed in Tehran Markets, Before and After of Treating by Modified Almond Shells

<table>
<thead>
<tr>
<th>Crop</th>
<th>n</th>
<th>Mean (NO₃⁻⁻) Before Treating by Almonds Shells Powder mg/kg ±S.E.*</th>
<th>Mean (NO₃⁻⁻) After Treating by Almonds Shells Powder mg/kg ±S.E.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broccoli (Brassica oleraces)</td>
<td>45</td>
<td>301.38 ±12 .44a</td>
<td>165.22± 9.43b</td>
</tr>
<tr>
<td>Cabbage</td>
<td>65</td>
<td>1039.64 ±39.49a</td>
<td>876.5 ± 23.89</td>
</tr>
<tr>
<td>Carrot</td>
<td>78</td>
<td>573.40±1.23</td>
<td>237.1±10.21</td>
</tr>
<tr>
<td>Celerie (Apium graveolens)</td>
<td>120</td>
<td>3628 ±43.4</td>
<td>2825 ± 50.6</td>
</tr>
<tr>
<td>Chinese cabbage</td>
<td>30</td>
<td>3899.5±54.01</td>
<td>2988.1±57.8</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>35</td>
<td>256.82±23.11</td>
<td>204.51±10.13</td>
</tr>
<tr>
<td>Eggplant</td>
<td>66</td>
<td>257.11±9.76</td>
<td>214.87± 10.3</td>
</tr>
<tr>
<td>Lettuce Romaine (Lactuca Sativa)</td>
<td>150</td>
<td>2433.8 ± 65.61</td>
<td>1001.8 ± 14.2</td>
</tr>
<tr>
<td>Lettuce Iceberg</td>
<td>120</td>
<td>2678.13±32.05</td>
<td>1011.7 ± 18.2</td>
</tr>
<tr>
<td>Potato</td>
<td>150</td>
<td>430.11 ± 12.3</td>
<td>237± 8.76</td>
</tr>
</tbody>
</table>

*S.E.: Standard Error of the mean

Mean in a column with different superscript alphabets differ significantly (P<0.05)
The findings from this research reveal that nitrate levels in untreated vegetable samples are significantly higher than those found in treated samples by almond shell as adsorbent \((P < 0.001)\) except in Cauliflower and eggplant crops which treated samples although have less nitrate content remarkably but the statistics shows insignificant reduction. It should be noticed that while nitrate is consumed in a normal diet containing studied vegetables and crops, other bioactive substances concomitantly consumed, such as the antioxidant vitamin C, which probably inhibit the endogenous formation of nitrosamines.

**Discussion**

Results from current study revealed that nitrite levels in untreated vegetable samples are significantly higher than those found in treated samples by almond shell as adsorbent \((P < 0.001)\) except in Cauliflower and eggplant crops which treated samples although have less nitrate content remarkably but the statistics shows insignificant reduction. It should be noticed that while nitrate is consumed in a normal diet containing studied vegetables and crops, other bioactive substances concomitantly consumed, such as the antioxidant vitamin C, which probably inhibit the endogenous formation of nitrosamines.

<table>
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<tr>
<th>Crop</th>
<th>n</th>
<th>Mean ((\text{NO}_3^-)) Before Treating by Almonds Shells Powder mg/kg ±S.E. *</th>
<th>Mean ((\text{NO}_3^-)) After Treating by Almonds Shells Powder mg/kg ±S.E. *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broccoli (Brassica oleraces)</td>
<td>45</td>
<td>0.73±0.18a</td>
<td>0.42±0.06a</td>
</tr>
<tr>
<td>Cabbage</td>
<td>65</td>
<td>1.65±0.49a</td>
<td>1.44±0.65a</td>
</tr>
<tr>
<td>Carrot</td>
<td>78</td>
<td>0.83±0.20a</td>
<td>0.56±0.09a</td>
</tr>
<tr>
<td>Celery (Apium graveolens)</td>
<td>120</td>
<td>3.10±0.18a</td>
<td>1.87±0.31a</td>
</tr>
<tr>
<td>Chinese cabbage</td>
<td>30</td>
<td>3.44±0.25a</td>
<td>2.03±0.56a</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>35</td>
<td>0.86±0.24a</td>
<td>0.65±0.16a</td>
</tr>
<tr>
<td>Eggplant</td>
<td>66</td>
<td>0.88±0.08a</td>
<td>0.36±0.05a</td>
</tr>
<tr>
<td>Lettuce Romaine (Lactuca Sativa)</td>
<td>150</td>
<td>0.86±0.03a</td>
<td>0.46±0.09a</td>
</tr>
<tr>
<td>Lettuce Iceberg</td>
<td>120</td>
<td>0.91±0.06a</td>
<td>0.49±0.04a</td>
</tr>
<tr>
<td>Potato</td>
<td>150</td>
<td>0.68±0.16a</td>
<td>0.62±0.18a</td>
</tr>
</tbody>
</table>

*S.E.: Standard Error of the mean

Mean in a column with different superscript alphabets differ significantly \((P < 0.05)\)
The nitrate levels for cabbage, iceberg and Romania Lettuces generally were below the allowable range. The nitrate content must be lower than 1 mg/kg [10, 15]. The nitrate content results in this survey were lower than 1 mg/kg, except in some celery and Spinach samples which were higher than 1 mg/kg but still in acceptable range and propose no danger on human health. It was reported that the nitrate levels start to be dangerous if it is higher than 100 mg/kg [10, 15] which is not the case here.

Due to the variability in nitrate and nitrite concentrations of foods reported in other studies [6-12, 15, 27-31], nitrate and nitrite analyses were conducted on mostly consumed vegetables in Iran. The findings from this research reveal that nitrate levels in leafy vegetables such as celery and iceberg and Romania lettuce were significantly higher than those found in root vegetables (P < 0.001). These data indicates that the average intake of nitrate from most of leafy vegetables is below the acceptable daily intake, i.e. 3/7 mg nitrate ion/kg body weight per day, but the total intake should be monitored to protect groups at risk, such as children and vegetarians.

Conclusion

When nitrate is consumed in a normal diet containing vegetables, other bioactive substances concomitantly consumed, such as the antioxidant vitamin C, may inhibit the endogenous formation of nitrosamines. However, high level consumers of vegetables grown under unfavorable local conditions may exceed the ADI by approximately two fold. In this report, the nitrate concentrations were not corrected for mitigating factors, such as fruit consumption and processing effect, which may overestimate exposure. The consumption of more than 45.1 g of contaminated lettuce or 38.9 g celery at the median nitrate concentration would lead to an excursion above the ADI without taking into account any other sources of nitrate exposure. Our finding proved that soaking vegetables after 15 minutes in vicinity of powder of modified shell of wild endemic almonds: A. lycioides and A. wendelboi significantly (P < 0.01) decreased nitrate and nitrite contents from most vegetables especially iceberg and Romania lettuce samples and (P < 0.03) for carrot samples. The results revealed that nitrate and nitrite contents of celery, Iceberg & Romani lettuce and carrot were decreased significantly (p<0.05). Different concentration of almonds ‘shell did not influence sensory attributes of vegetables. The most efficient time for treating by powder of shells was 15 min, but the most practical one is recommended 20 min.

Addition of almonds ‘shell did not change pH of all studied vegetable samples. Application of modified shell of wild almond suggested as a novel, safe and economic method for removal of nitrate in contaminated crops and vegetables.

In accordance to the results we obtained, we highly recommend that more work to be conducted on this subject. Both governmental and nongovernmental organizations have to focus on this issue and must consider it as part of their strategy. The education and research centers have to perform more comprehensive survey on other agricultural crops and on different production areas and of course in other adsorbents. The aim of this work was mainly to highlight this important and crucial issue which has a direct effect on our health and environment.

Acknowledgement

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Conflict of Interests

It is declared that the authors neither have any financial gain nor conflict of interests regarding this paper.

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