Effects of vegetables pretreatment in safe solutions: nitrate loss in raw vegetables and preserving their freshness

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Abstract:

In this study, we try to evaluate the effects of different pretreatments on nitrate content in many vegetables. Three edible solutions, including NaCl salt, baking soda ($NaHCO_3$) and vinegar were used as nitrate losing agents for leafy vegetables (mint, basil, savory, tarragon, parsley, coriander, cress, radish and chives) and warm water was used for root vegetables (potato, onion and carrot). Different patterns of change in percentage of nitrate vegetables was observed by treatment in these solutions. However, pretreatment in vinegar solution decreased nitrate percentage in all cases (14.6 - 41 (gr/100gr)). Pretreatment in warm water decreased the nitrate percentage in potato and onion in wide range (26.6 -68.6 (gr/100gr)), but a considerable increase was observed in carrot (125.9 (gr/100gr)). Results showed that treatment in the vinegar solution and warm water led to decrease in nitrate content of vegetables, while the freshness and safety of vegetables were preserved.

Keywords: Leafy Vegetables, Potato, Onion, Pretreatment, Nitrate.

1. Introduction:

Vegetables play a vital role in human diets, as they support the normal functioning of different body systems. They do so by providing our cells with vitamins, minerals, fiber, essential oil and phytonutrients. Their consumption is encouraged in many countries by government health agencies to protect our body against a range of illnesses. Regular consumption of vegetables is associated with a reduced risk of cardiovascular disease, stroke and certain cancers (Van Duyan & Pivonka, 2000). Some vegetables such as salad are eaten raw so that their natural taste is retained, and heat labile nutrients is preserved. On the other hand, vegetables can become contaminated by inorganic ions such as nitrate and nitrite, which can be a threat for human's health. Vegetables are known as the major source of nitrate and nitrite intake in the human diet (Amr & Hadidi, 2001). It has been estimated that 75–80% of the total daily intake comes from vegetables (Dennis & Wilson, 2003). Several factors can potentially influence the level of nitrate and nitrite in various raw vegetables. These factors include type, amount and form of nitrogen fertilizer (Elia, Santamaria, & Serio, 1998; Lips, Leidi, Siberbush, Soares, & Lewis, 1990) high levels of organic matter in the soil, growth-depressing temperatures (Habben, 1973) and geographical latitude (due to supplemental light usage).

Nitrite is believed to contribute to some forms of cancer (Cassens, 1997). It is also implicated in methaemoglobinaemia (Chan, 2011). Although nitrate is relatively harmless by itself, it is a major precursor of nitrite in the body. It is estimated that around 5% of ingested nitrate is reduced to nitrite by micro-organisms in the saliva (Cassens, 1995). It has been reported that there is a relationship between existing nitrate in consumed food and the increase of cancer risk (Mitacek, 2008). Nitrate levels can be significant in many leafy vegetables such as lettuce (Lactuca sativa L.), spinach (Spinacia oleracea L.), celery (Apium graveolens L.), and marjoram (Majorana hortensis, Moench) (Samuoliene, 2009). Therefore, the World Health Organization (WHO) has

set an acceptable daily intake (ADI) for nitrate of 3.7 mg kg^{-1} body weight (Speijers & Van Den Brandt, 2003). So, controlling the nitrate percentage in vegetables and finding methods to reduce nitrate amounts in vegetables before consumption seems necessary. This can be reached in two different ways: before harvesting the products or after harvesting, but before consumption. In the first case, research shows that the nitrates levels from organic production varied between 1.45 and 6.40 mg/kg fresh weight (FW), whereas those from conventional production ranged from 10.5 to 45.19 mg/kg FW (Aires, 2013). Light intensity at time of harvest is another parameter which can affect the vegetable nitrate content in farm and before harvesting. The results of research showed nitrate and nitrite concentrations varied significantly over the 24 h period and appeared to be related to changes in light intensity (Chang, 2013; Rouphael, 2016). The effect of different pre-treatment processes such as boiling, baking and frying on nitrate percentage in vegetables was investigated. Nitrate percentage of fresh root vegetables ranges from 53.76 to 258.00 $mgkg^{-1}$. Boiling reduces nitrate percentage by 23.30–42.62%; frying in soya bean oil elevates nitrate percentage from 204.53–299.12%; Baking either did not change nitrate contents percentage or changed it slightly, 2.80–8.43% (Prasad, 2009). Changes in the levels of broccoli nitrate percentage in the freezing storage and cooking processes were also determined. Industrial freezing rose the nitrate levels (127-232 ppm KNO_3), probably as the result of high levels in the processing water. Cooking decreased nitrate levels (Between 22 and 79%), but there were no differences in the nitrate levels of fresh and frozen vegetables (Astiasarin, 1996). Also, changes in nitrate percentage in leafy vegetables by microwave boiling with normal (BNW) and 5% NaCl solution (BSW) was investigated. It was observed that boiling process reduced nitrate percentage from fresh sample (4.5–73.6% by BSW and 22.5–98.8% by BNW). The study revealed differential pattern of change in nitrate percentage in vegetables by microwave boiling which will help in devising efficient cooking practices and contribute to health and nutritional security (Singh,

2015). However, cooking destroys essential nutrients such as vitamin C and kills vital enzymes that help your body to digest such foods. So, finding a pretreatment, which can reduce the nitrate percentage in raw vegetables and preserving the freshness of vegetables, seems necessary.

In this study, we tried to investigate nitrate losing percentage by treatment in different edible and safe solutions such as NaCl salt, baking soda ($NaHCO_3$) and vinegar, which are available easily. Thus, the effects of NaCl salt, baking soda ($NaHCO_3$) and vinegar solutions, with different concentrations and different times of leafy vegetables keeping in these solutions, on the nitrate percentage of leafy vegetables (nine type of leafy vegetables) was investigated. Regarding potato, onion and carrot, which are root vegetables, the effects of different retention times of these vegetables (potato and onion) in warm water on their nitrate percentage was investigated.

2. Experimental

2. 1. Chemical reagents, solvents and equipment

All the reagents used in this study were of analytical-reagent grade: Acetonitrile solvent was of HPLC grade (Pub Chem CID:6342), K_2HPO_4 salt (Pub Chem CID:24450), $K_4[Fe(CN)_6].3\ H_2O$ (Pub Chem CID:161067) , $Zn(CH_3COO)_2.2\ H_2O$ (Pub Chem CID:11192) and baking soda ($NaHCO_3$, Pub Chem CID:516892) were prepared from Merck company; sodium chloride (NaCl) and grape vinegar were prepared from Sanandaj city supermarkets. Different types of vegetables (mint, basil, savory, tarragon, parsley, coriander, cress, radish, chives, potato, onion and carrot) were prepared from vegetable markets in Sanandaj in the west of Iran. These vegetables were divided into 4 groups, mint and basil as group 1, parsley and coriander as group 2, savory and tarragon as group 3 and cress, radish, chives as group 4.

2. 1. 1. Carrez solution No. 1

150 g of potassium hexacyanoferrate (II) ($K_4[Fe(CN)_6].3\ H_2O$) is dissolved in water, and then is mixed well and diluted to 1000 ml with water. It is stored in a brown bottle and is replaced every week.

2. 1. 2. Carrez solution No. 2

220 g of zinc acetate ($Zn(CH_3COO)_2.2\ H_2O$) is dissolved in water, and then 30 ml of glacial acetic acid is added, mixed and diluted to 1000 ml with water.

2. 1. 3. Equipment

In order to collect and process data, analyses were performed with HPLC knuwer S series equipped with G1322A degasser, G1311A quaternary pump, S 2600 detector and HP ChemStation.

2. 2. Pretreatment methods

Here, two different types of vegetables, including leafy and root vegetables, were evaluated.

2. 2. 1. Leafy vegetables

In terms of leafy vegetables, rotten leaves and non – edible parts of vegetables are removed, at first vegetables are rinsed with tap water in order to remove soil particles from the surface of them. Three different edible solutions, including baking soda ($NaHCO_3$), sodium chloride (NaCl) and grape vinegar are used as nitrate losing agents in vegetables matrix.

2. 2. 1. 1. Baking soda ($NaHCO_3$)

Baking soda solution with two different concentrations, 5 and 10 percentage were prepared with tap water. Then, rinsed vegetables were kept in each of the prepared solutions for 10, 20 and 30 minutes. In the final step, vegetables were rinsed with tap water and prepared for nitrate extraction and analysis.

2. 2. 1. 2. Sodium chloride (NaCl)

In this section, similar to 2. 2. 1. 1, solutions of sodium chloride salt with two different concentrations 2 and 5 percentage were prepared with tap water, and then rinsed vegetables were kept in each of the prepared solutions for 10, 20 and 30 minutes. As the final step, vegetables were rinsed with tap water and prepared for nitrate extraction and analysis.

2. 2. 1. 3. Grape vinegar

Similar to above two sections, two different concentration of grape vinegar 10 and 20 percentage were prepared with tap water and after that rinsed vegetables were kept in each of the prepared solutions for 10, 20 and 30 minutes. As the concluding step, vegetables were rinsed with tap water and prepared for nitrate extraction and analysis.

At each of the above steps, vegetables which kept in tap water for 10, 20 and 30 minutes were analyzed as control group.

2. 2. 2. Root vegetables

Concerning root vegetables such as potato, onion and carrot, which have tighter texture relative to leafy vegetables, warm water was used as nitrate losing agent. For this purpose, after removing non edible parts of these vegetables, chopping them in smaller pieces, and rinsing them with tap water to remove soil particles from the surface of vegetable pieces, they were kept in warm water for 3, 7 and 10 minutes. So, rinsed vegetable pieces were prepared for nitrate extraction and analysis. In these cases, vegetable pieces, which kept in tap water for different times 10, 20 and 30 minutes, were analyzed as control group.

2. 3. Nitrate extraction and analysis

The standard method (BS EN 12014-2: 1997) was used for nitrate extraction and analysis. Briefly in this method, a representative sample was taken. Then, the sample was shred in a laboratory cutter, and was thoroughly homogenized using a homogenizer. After that, a portion (not less than 10 g) of the sample was kept in a 100 ml flask, and then approximately 90 ml of hot water was

added to it, standing the flask in a boiling water bath for 15 min. The flask was cooled down to room temperature and then the flask was agitated. Following that, the entire solution was filtered through a fluted filter paper so as to clarify and purify the filtrate, i.e. the sample solution. Pipette 5 ml of each sample solution into a 15 ml falkon, add 2.5 ml of Carrez solution No. 1, mix, add 2.5 ml of Carrez solution No. 2 shake thoroughly and centrifuged at 8000 (rpm) for 10 min then filter a portion of the upper suspension through a PVDF syringe filter 0.45 μm. Finally 20 μl of the solution was injected to the HPLC instrument. The mobile phase is the mixture of K_2HPO_4 solution pH=3 (95%) and acetonitrile solvent (5%). Wavelength of detector was set at 205 nm and measurements were done in room temperature. LiChrosorb-NH_2 column with a particle size of 5 μm (at least 250 mm long), internal diameter (i.d.) 46 mm, and a precolumn with the same packing were used as separating column.

2. 4. Statistical analysis

The triplicate data from experiment were analyzed for ANOVA. The mean, range, standard deviation and percentage changes in the nitrate were calculated by Microsoft Excel Window 2010.

3. Results and discussion:

3.1 Nitrate changes in vegetables after different pretreatments

The mean value nitrate analysis of 12 fresh vegetables from different regions in Iran are presented in Table 1. According to the Table 1, nitrate content in these vegetables has a wide range, 1173.3 to 2108.71 ppm in leafy vegetables and 108 to 422 ppm in root vegetables. In this study, according to vegetable tissue, two different pretreatment for decreasing the nitrate percentage in these vegetables before consumption were investigated. With regard to leafy vegetables, three edible solutions including vinegar, baking soda, and sodium chloride salt as

nitrate reducing agent with different pH and ionic strength were used, but warm water was used for root vegetables.

3. 1. 1 Nitrate changing in leafy vegetables

With regard to leafy vegetables, three edible solutions, namely vinegar, backing soda and sodium chloride salt as nitrate losing agents were employed. At first, the effect of different concentrations of above solutions on nitrate percentage of different leafy vegetable groups was investigated (data not shown). Effective concentrations are baking soda 10 (gr/100gr), NaCl salt 5 (gr/100gr) and vinegar 20 (gr/100gr). So the effect of optimum concentration was compared. Results showed in (Figure 1), according to obtained results, the sodium chloride salt solution increases the nitrate content of vegetables considerably in wide range, from 20.25 (gr/100gr) in group 4 to 85 (gr/100gr) in group 1, but this solution decreases nitrate content 12.4 (gr/100gr) in group 3. Keeping in backing soda increases nitrate content in group 1, about 33(gr/100gr). For other groups, however, nitrate content decreased partially: 0.67 (gr/100gr) in group 2, 6.2 (gr/100gr) in group 3 and 0.22 (gr/100gr) in group 4. Finally, the results showed that vinegar solution decreased nitrate content in all groups considerably from 14.6 (gr/100gr) in group 2 to 41 (gr/100gr) in group 3. As a result, vinegar (20 ml/100ml) solution can be offered as the best losing agent to decrease nitrate in raw leafy vegetables before consumption. In the next step, keeping time of vegetables in vinegar (20 ml/100ml) solution should be optimized. For this purpose, the mixture of all groups of vegetables kept in vinegar (20 ml/100ml) solution for different times and nitrate content of vegetables were measured after 10, 20 and 30 minutes. Based on Figure 2, it can be observed that after keeping the mix of all groups of vegetables for 10 and 30 minutes in vinegar (20 ml/100ml) solution, nitrate content decreased to 24 and 26 (gr/100gr), respectively. Also, keeping the vegetables in vinegar solution for 30 min caused flattering the vegetables. In order to preserve the freshness of the vegetables, 10 minutes was

considered as optimized time for keeping the vegetables in this solution. Finally, to confirm the important role of vinegar in vegetables nitrate loss, another experiment was done. In this experiment, the mixture of all vegetable groups after rinsing with water kept in water for 30 minutes, and simultaneously similar sample kept in vinegar (20 gr/100gr) for 10 minutes. Therefore, the nitrate content of both was measured. Results shown in figure 3 indicated that vegetable nitrate after keeping in water for 30 minutes is equal to of nitrate content in rinsed vegetables and keeping the vegetables in water does not lead to nitrate loss. However, nitrate content in another sample kept in vinegar (20 gr/100gr) for 10 minutes decreased compared to the sample which only rinsed with water. This can be attributed to the enhanced softening of the cell wall at low pH (Doesburg, (1965) & Van Buren, (1979)). In other words, vinegar by decreasing the environmental pH enhanced the softness of the cell wall and cause the extract of the nitrate ions from vegetable cells. In this study, we tried to preserve the freshness of vegetables by controlling the time of keeping the vegetables in a vinegar solution, while the nitrate content in vegetables decreased.

3.1.2. Nitrate changing in root vegetables

In this section, the effects of keeping the root vegetables in warm water on the nitrate content of these vegetables was investigated. Results showed (Figure 4) that keeping the onion in warm water for 3 minutes, decreases nitrate content to 68.6 (gr/100gr) and for 10 minutes decreased it to approximately about 54 (gr/100gr). So, regarding onion, 3 minutes retention in warm water can be chosen as the optimum time for nitrate loss. Also, according to Figure 3, highest decrease in potato nitrate content was observed after keeping potato pieces in warm water for 10 minutes (26.6 (gr/100gr)). Nevertheless, with regard to carrot, keeping in warm water for 3 to 10 min resulted in increase the nitrate percentage from 61 to 125 (gr/100gr), so only rinsing with cold water is recommended.

4. Conclusion:

Compared to rinsing with water, pretreatment in NaCl salt solution increased vegetable nitrate considerably due to increase in ionic strength and reverse osmosis. Baking soda solution decreased vegetable nitrate partially, but in vinegar solution, vegetable nitrate decreased considerably. Keeping the mixture of all vegetables in vinegar solution (20 gr/100gr) for 10 min decreased nitrate considerably. In the case of root vegetables, including onion and potato, keeping in warm water for 3 and 10 min decreased nitrate percentage 68 and 26 (gr/100gr) respectively. However, in terms of carrot, keeping in warm water led to a considerable increase in nitrate. By these pretreatments, vegetable nitrate decreased without the destruction of vegetable tissues and their freshness preserved. This is considered as the advantage of this study compared to the previous studies which tried decrease nitrate in vegetables by cooking, boiling or freezing. Furthermore, vinegar is edible and safe, so in addition to decreasing the vegetable nitrate, their safety preserved.

Acknowledgment:

This research was supported by the Vice Chancellor for Research and Technology of the Kurdistan University of Medical Sciences. Authors thanked Vice Chancellor for Research and Technology for financial support.

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Figures caption:

Figure 1. Nitrate percent changing of leafy vegetables after keeping them in vinegar, backing soda and sodium chloride salt.

Figure 2. Nitrate changing of the leafy vegetables mix after keeping them in vinegar (20 gr/100gr) for different times.

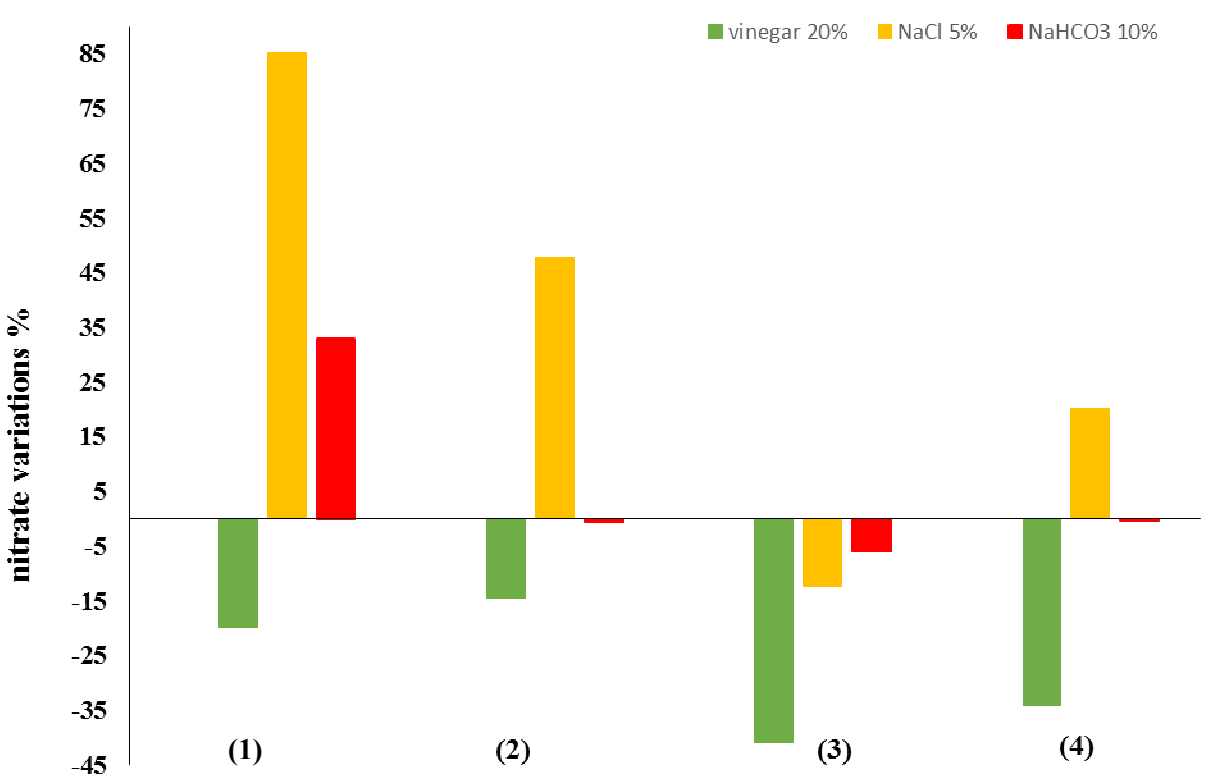
Figure 3. Nitrate content in water rinsing vegetables, vegetables kept in water for 30 minutes and vegetables kept in vinegar (20 gr/100gr) for 10 minutes.

Figure 4. Nitrate changing in onion, potato and carrot after keeping in warm water for different times 0 minute (means rinsing with cold water only), 3 minutes and 10 minutes.

Table 1. Nitrate in 12 fresh vegetables of different regions in Iran.

Vegetables	Edible parts	Nitrate (ppm)
1	Leaves	2001.89 (± 50.72)
2	Leaves	1961.41 (±31)
3	Leaves	2108.71 (± 77)
4	Leaves	1173.3 (±76.9)
5	Root	108.31 (±12.95)
6	Root	234.79 (±1.98)
7	Root	422.6 (± 16)

1. Group 1 of leafy vegetables, 2. Group 2 of leafy vegetables, 3. Group 3 of leafy vegetables, 4. Group 4 of leafy vegetables, 5. Potato, 6. Onion, 7. Carrot.


vinegar 20%
NaCl 5%
NaHCO3 10%
nitrate variations %
85
75
65
55
45
35
25
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-25
-35
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Figure 1.

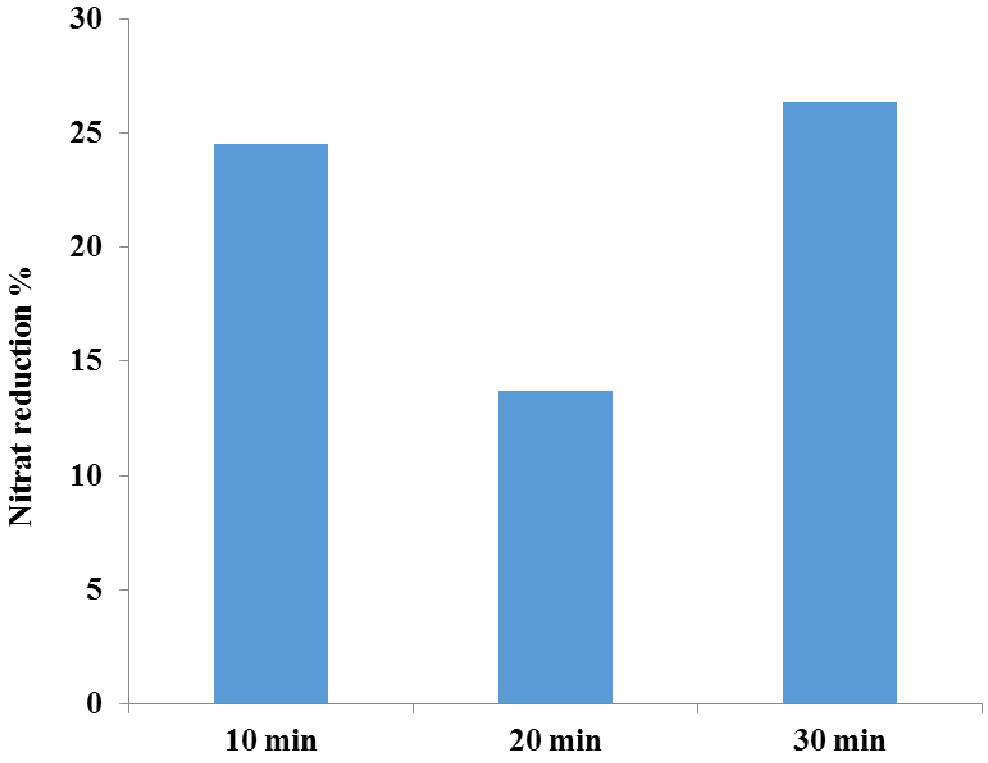

Figure 2.

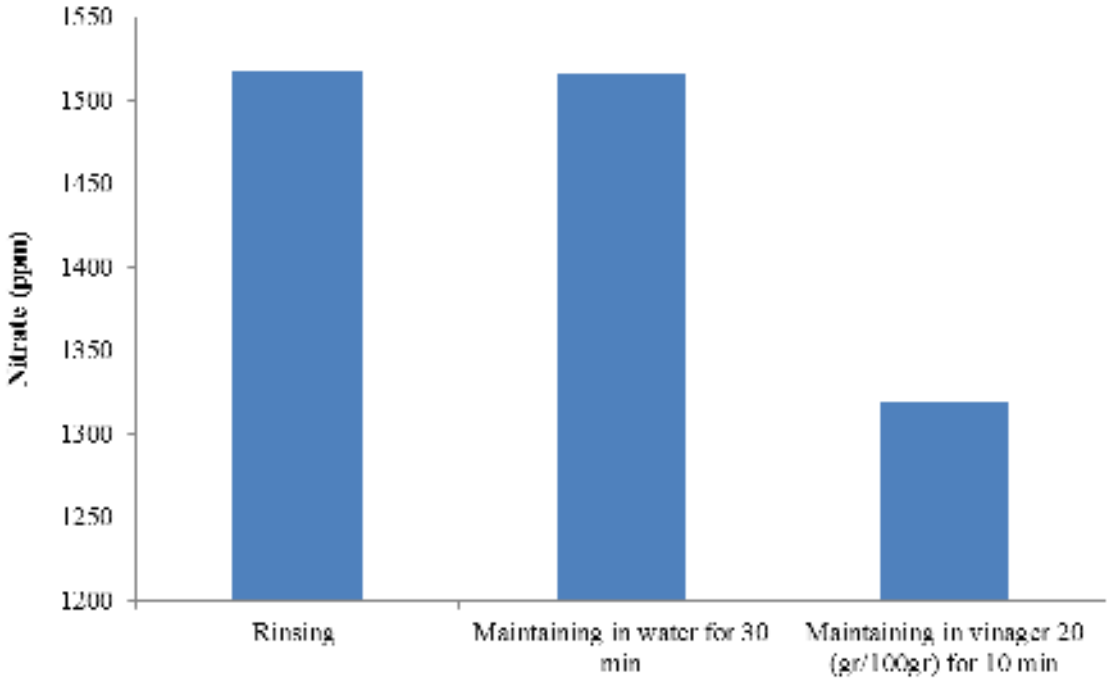
1550
1500
1450
1400
1350
1300
1250
1200
Nitrate (ppm)
Rinsing
Maintaining in water for 30 min
Maintaining in vinagar 20 (gr/100gr) for 10 min

Figure 3.

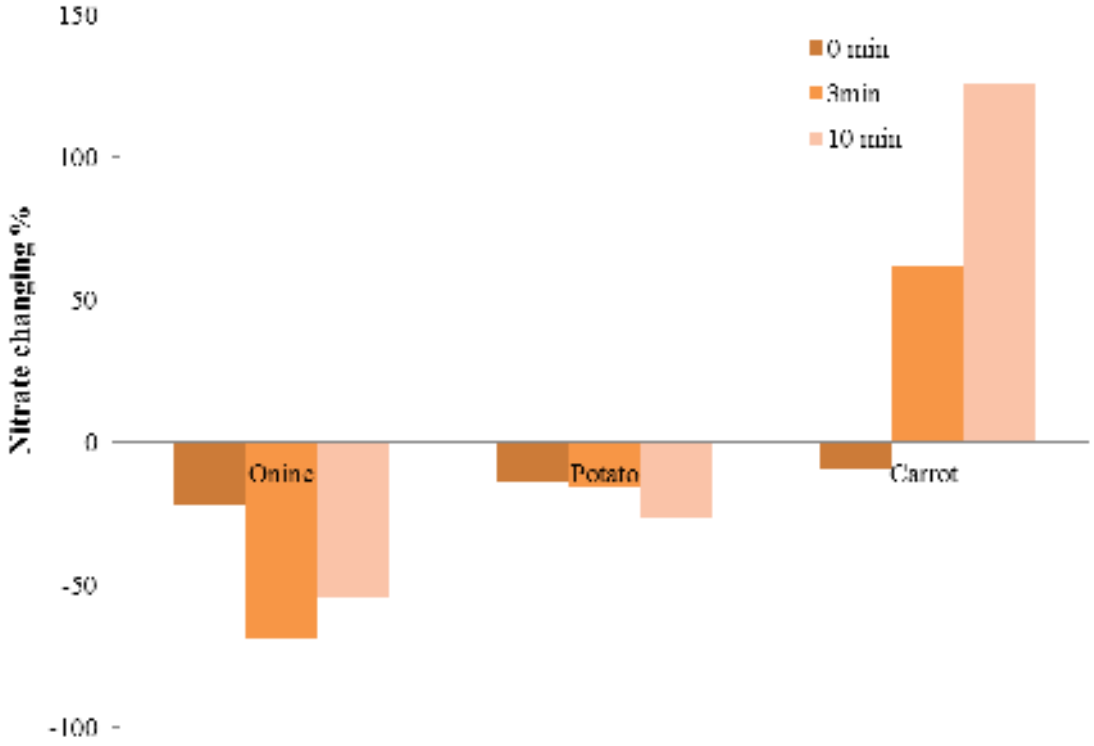
150
100
50
0
-50
-100
Nitrate changing %
0 min
3min
10 min
Onion
Potato
Carrot

Figure 4.

Highlights

- Effects of different pretreatments on nitrate content in many leafy and root vegetables were investigated.
- Three edible solutions, including NaCl salt, baking soda ($NaHCO_3$) and vinegar were used as nitrate losing agents for leafy vegetables, and warm water was used for root vegetables.
- Pretreatment in vinegar solution with concentration 20 (gr/100gr) for 10 minutes decreased nitrate percentage in leafy vegetables (14.6 - 41 (gr/100gr)), while the freshness and safety of vegetables were preserved.
- Pretreatment in warm water decreased the nitrate percentage in root vegetables, including potato and onion in wide range (26.6 -68.6 (gr/100gr)).