International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.9, No.11 pp 218-223, 2016

The Effect of Boiling on Nitrate and Nitrite Contents in Celery (*Apium graveolens L.*)

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Abstract : The purpose of this study was to determine the effect of boiling using demineral and mineral water on the contents of nitrate and nitrite in celery.
Celery used in this study was purchased from a local market at Pajak Sore Padang Bulan Medan. Celery was boiled with varying boiling time, 2, 4 and 6 minutes using demineral water (distilled water) and mineral water (tap water). Nitrite was identified with sulfanilic acid reagent and N-(1-naphthyl) ethylenediamine dihydrochloride (NED). Identification of nitrate was done by ferrous sulfate solution and concentrated sulfuric acid. Determination of nitrite was conducted by visible spectrophotometer using a coloring reagent NED at maximum wavelength of 540 nm. Nitrate was determined after reduction into nitrite and then analyzed as nitrite.
The results showed that the boiling time and the type of water used affecting levels of nitrate and nitrite in celery. Levels of nitrate and nitrite in fresh celery were 52.17 mg/kg and 25.57 mg/kg respectively. Levels of nitrate and nitrite were changed during boiling process; and the type of water was also influential. Levels of nitrite after boiling process for 6 minutes using demineral water decreased from initial level (25.57 mg/kg) to 11.86 mg/kg; while nitrate level decreased from 52.17 to 16.35 mg/kg. Levels of nitrite after boiling process for 6 minutes using tap water decreased from initial level (25.57 mg/kg) to 13.39 mg/kg; while nitrate level decreased from 52.17 to 22.20 mg/kg. The nitrite and nitrate contents in celery which boiled with demineral water differently affected; nitrate decreased from 52.17 mg/kg to 16.34 mg/kg (68.6%) and nitrite from 25.57 mg /kg to 11.85 mg /kg (54. 2%), while boiling with mineral water on nitrate decreased from 52.17 mg / kg to 22.20 mg/kg (57%) and the nitrite from 25.57 mg/kg to 13.39 mg / kg (48%).
The effect of boiling using demineral water was more effective to decrease nitrate and nitrite than using mineral water.
Keywords: celery, boiling, nitrite, nitrate, visible spectrophotometer.

Introduction

Vegetables contain nitrite and nitrate that may cause negative effects for health[1]. Nitrate contents in vegetables ranging from 1 to 10.000 mg/kg wet base. While nitrite in fresh vegetables is usually low that is about 2 mg/kg. Vegetables like lettuce and celery contain nitrate 13000 and 5300 mg/kg respectively[2,3].

Nitrate is necessary in vegetables for good growth and the synthesis of biological components and particularly protein. Nitrate and nitrite present in environmental as well as in vegetables. Nitrate in vegetables can be converted into nitrite by bacteria and enzyme. Nitrate can be metabolized by nitrate reductase enzyme into nitrite [3,4]. The Acceptable Daily Intake (ADI) by FAO/WHO is 220 mg/kg for nitrate and 8 mg/kg for nitrite for man with body weight of 60 kg. Nitrite may cause methaemoglobinemia and may react with alkylamine mainly dimethylamine and trimethylamine to form carcinogen nitrosamines[5,6]. Human exposure to nitrosamines may occur through endogenous and exogenous sources from foods and beverages leading to increase the risk of gastric, esophageal, nasopharyngeal, and bladder cancer. There is conclusive evidence that nitrosamine is a potent carcinogen in experimental animals by several routes [7,8,9].

There are several factors affecting nitrate and nitrite contents in vegetables including genetic, environmental, atmosphere humidity, fertilization, storage condition and processing procedure etc[10]. Boiling process found to decrease nitrate and nitrite in vegetables[11], but storage at cold temperature (10^0C) and ambient temperature ($\pm 28^0$C) increased nitrate and nitrite contents in lettuce[12]. The aim of this study was to investigate the effect of boiling process using mineral and demineral water on the nitrite and nitrate levels in celery *(Apium graveolens L.).*

Materials and Method

Materials

Celery used was purchased from local market at Pajak Sore Padang Bulan in Medan. Chemicals used were pro analysis grade product of E. Merck (Germany) including; N-(1-naftil) etylendiamin dihydrochloride (NED), sodium nitrite, sulfanilic acid, glacial acetic acid, hydrochloric acid, ferrous sulfate, concentrated sulfuric acid, zincum powder, demineral water (distilled water) and mineral water (tap water).

Boiling Process

Fresh celery (± 50 g) was placed in separated stainless-steel pan using two types of water, that was demineral water (distilled water) and mineral water (tap water) about 500 ml. Boiling process was carried out for 2, 4 and 6 minutes. This process was assumed to be similar to the normal cooking done in households.

Instruments

Instruments used in this study were spectrophotometer UV-Vis (UV- 1800 Shimadzu), analytical balance (Boeco Germany), water bath, filter paper, thermometer, stirring rod and glass wares.

Preparation of Reagents

Preparation of reagents used was conducted as described in AOAC [12]. Acetic acid solution of 15% was prepared by dissolving 75 ml glacial acetic acid and diluted with distilled water to make 500 ml. NED solution was made by dissolving 0.350 g N-(1-naftil) etylendiamin dihydrochloride in 250 ml acetic acid solution 15%, filtered and stored in brown bottle. Sulfanilic acid solution was prepared by dissolving 0.850 g sulfanilic acid in 250 ml acetic solution of 15%, filtered and stored in brown bottle [13].

Preparation of nitrite standard stock solution

The amount of 100 mg sodium nitrite was transferred into 100 ml volumetric flask and dissolved in distilled water, and then made to 100 ml (1000 μg/ml) (Solution I). From this solution 1 ml was pipetted into 100 ml volumetric flask and added distilled water to make 100 ml (10 μg/ml) (Solution II) [14].

Identification of Nitrite and Nitrate

About 10 g ground sample was transferred in to a beaker glass, added 150 ml distilled water, heated on water bath and shaken for a little while, then cooled and supernatant was collected and placed in a test tube for nitrite and nitrate identification. Presence of nitrite was identified with sulfanilic acid and NED solution and allowed for a little while. The appearance of violet color was to indicate the presence of nitrite [15]. The presence of nitrate was conducted by adding several drops ferrous sulfate solution and then few drops of concentrated sulfuric acid added slowly through the inner wall of the test tube. The presence of nitrate was indicated by the appearance of chocolate ring [16].

Determination of absorbance curve of nitrite standard solution

Four (4) ml of stock standard solution II was transferred into 50 ml volumetric flask, added 2.5 ml of sulfanilic acid solution and shaken, after 5 minutes 2.5 ml NED reagent was added and made to volume with distilled water and homogenized (0.8 µg/ml). Absorbance was measured at wave length of 400-800 nm. Then absorbance and wave length was plotted automatically to construct absorbance curve. Wave length of maximum absorbance was determined from the absorbance curve. The maximum absorbance of standard solution was found at the wave length of 540 nm used in the analysis [14].

Absorbance stability of derivatized nitrite to determine best working time

Four (4) mL stock solution II transferred into volumetric flask of 50 ml, to which 2.5 ml of sulfanilic acid solution was added and stirred. After 5 minutes, 2.5 ml NED reagent solution was added then distilled water was added to make 50 ml. Absorbance was measured at wave-length of maximum absorbance (540 nm), and stability of absorbance was determined by observing absorbance at every minute for 1 hour. The absorbance was found to be relatively stable during period after ten to fourteen minutes (10-14 minutes) [14].

Determination of calibration curve

Standard stock solution II (10 µg/ml) of different volumes (2 ml; 3 ml; 4 ml; 5 ml, and 6 ml) were transferred into different volumetric flasks of 50 ml, then 2.5 ml sulfanilic acid reagent added and stirred to homogenize. After 5 minutes, 2.5 ml NED reagent was added, then distilled water was added to make volume of 50 ml and homogenized. The concentrations of prepared solutions were of 0.4 µg/ml; 0.6 µg/ml; 0.8 µg/ml; 1.0 µg/ml; 1.2 µg/ml. Absorbance of each solution was measured at wave-length of 540 nm after about 12 minutes. Calibration curve was constructed by plotting absorbance versus concentration of each solution. From the graph obtained, then linearity of regression equation and correlation coefficient were calculated. [13-14]. From the experiment was found that regression equation was Y=0.55 X + 0.009, and correlation coefficient was 0.999 (R^2=0.999).

Determination of Nitrite and Nitrate in Celery

Determination of Nitrite and Nitrate levels in vegetables was carried out according to the procedure described in *Association Official Methods Of Analytical Chemists* (AOAC) XVII edition [13]. Ten (10) gram ground sample was transferred into 250 ml beaker glass. Then, hot distilled water (± 80ºC) was added to make volume of 150 ml. Homogenized by stirring and heated on water-bath for 15 minutes while stirring. Allowed to cool and then transferred quantitatively into a volumetric flask of 250 ml. Added distilled water to make volume of 250 ml, then filtered. About 10 ml first filtrate was discarded, the following filtrate was collected. The filtrate obtained was used firstly for nitrite and then for nitrate quantification. For nitrite determination; ten (10 ml) of filtrate transferred into a volumetric flask of 50 ml, then 2.5 ml sulfanilic acid reagent was added and stirred. After 5 minutes, 2.5 ml reagent NED was added, then distilled water was added to make 50 ml, and then homogenized. Absorbance was measured at wave-length of 540 nm after period of 10 to 14 minutes time.

Determination of nitrate was done after reducing nitrate into nitrite and determined as nitrite [16]. Ten (10) ml of filtrate prepared above was transferred into a volumetric flask of 50 ml added Zn powder (1 g), shaken and allowed to stand for 10 minutes, then 2.5 ml sulfanilic acid reagent was added and stirred to homogenous. After 5 minutes, 2.5 ml reagent NED was added, then distilled water was added to make 50 ml, then homogenized.[14,16]. Absorbance was measured at wave-length of 540 nm after period of 10 to 14 minutes time. The absorbance measured in this step was resulted from total nitrite (initial nitrite plus nitrite from reducing nitrate into nitrite). Dilution factor was 5 (10 ml filtrate to 50 ml). Level of nitrite in diluted sample filtrate was calculated from the regression equation obtained from calibration curve: Y=0.55 X + 0.009

Nitrite concentration was calculated:

$$C = \frac{X \times V \times Fp}{\text{Sample weigth (g)}}$$

Notes:
Y = Absorbance
C = concentration of nitrite in sample (µg/g)
X = Nitrite concentration in diluted sample solution (µg/g)
V = volume of sample solution before dilution (ml)
Fp = dilution factor

Concentration of nitrite from the reduction of nitrate into nitrite = concentration of total nitrite after reduction – concentration of nitrite before reduction.

Nitrate concentration = nitrite concentration after reduction x $\frac{MW\ nitrate}{MW\ nitrite}$

Rersults and Discussion

Identification of Nitrite and Nitrate in Celery

From identification tests it was proved that nitrite and nitrate were present in celery. Based on the test conducted after boiling with various time, the levels of nitrite and nitrate were found to decrease indicated by the decreasing intensity of color produced by the test conducted before and after boiling.

Absorption Curve of Nitrite and Determination of Working Time

Absorption curve was determined by measuring absorbance of standard solution of 0.8 µg/ml after derivatization with NED reagent. Measurement was carried out using wave length ranging from 400-800 nm. From the absorption curve found that maximum absorption of standard solution was found to be at 540 nm and this result is similar to that mentioned in AOAC[13]. This wave length was used to determine the appropriate working time by measuring absorbance of standard solution of 0.8µg/ml for 60 minutes and absorbance was found to be relatively stable about five minutes (after 10 to 14 minutes). So that working time was 5 minutes (after 10 to 14 minutes during measurement).

The Effect of Boiling on Nitrite and Nitrate Contents in Celery

The effect of boiling time using different water on nitrite and nitrate contents in celery is presented in Table 1 and Figure 1. From Table 1 and Figure 1 shown that nitrate content (52.17 mg/kg) was higher than nitrite (25.57 mg/kg), since more than 90% of the nitrogen absorbed by plants in the form of nitrate, and in addition nitrate is necessary for plant growth and to produce protein. The level of nitrite was much higher than usually present at the level of 1-5 mg/kg in most vegetables[3]. The results showed that the boiling time and the type of water used affecting levels of nitrate and nitrite in celery. As shown in Table 1 and Figure 1 that time and the type of water used in boiling process decreased the nitrate and nitrite contents in celery. The longer the boiling the lower the nitrite and nitrate contents would be in celery.

Table 1. The effect of boiling time using different water on nitrite and nitrate contents in celery

Boiling Process	Boiling time (min)	Nitrite content (mg/kg)	Nitrate content (mg/kg)
Boiling with mineral water (tap water)	0	25.57 ± 0.28	52.17 ± 2.02
	2	21.31 ± 0.31	32.73 ± 2.85
	4	15.41 ± 0.14	24.75 ± 1.37
	6	13.39± 0.65	22.20 ± 0.54
Boiling with demineral water (distilled water)	0	25.57 ± 0.28	52.17 ± 2.02
	2	15.46 ± 0.92	31.00 ± 0.78
	4	12.76 ± 0.41	25.40± 0.51
	6	11.86 ± 0.13	16.35 ± 1.64

Data given is the mean of 6 replicates ± Standard deviation

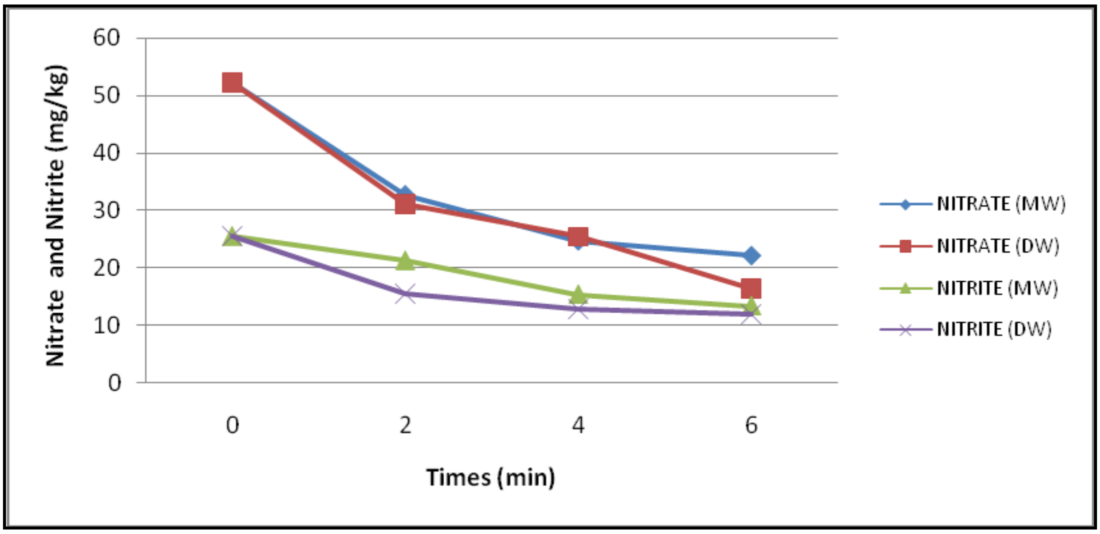

Figure 1. The effect of boiling using demineral water (DW) and mineral water (MW) on nitrate and nitrite contents in celery

The type of water used in boiling also affected differently and decreased nitrite and nitrate contents in celery. The boiling with demineral water was more effective than tap water to lower nitrate and nitrite contents because demineral water is more aggressive to dissolve nitrate and nitrite due to the absence of dissolved solute or especially the absence of mineral in the demineral water [17-18]. The boiling after 6 minutes with demineral water decreased nitrite from 25.5 to 11.8 mg/kg (54.2%) while with tap water changed from 25.5 to 13.4mg/kg (48%). Boiling with demineral water, the nitrate level changed from 52.17 mg/kg to 16.35 mg/kg (68%) while with tap water changed from 52.17 mg/kg to 22.20 mg/kg (57%). The result of this study was similar to the findings in previous researchers who reported that the nitrite and nitrate content lost in the range of 25% to 60% by boiling using water because nitrate and nitrite migrate in to the water used [11,19]. Therefore the nitrate and nitrite contents increased in the water used after boiling as it was also observed in this present study.

The nitrite and nitrate content of waters before and after boiling in this experiment was also measured. Demineral water before boiling did not contain nitrite and nitrate, while tap water contained 11.9 mg/l nitrite and 2.71 mg/l nitrite. The nitrate and nitrite content of tap water after boiling increased to 41.45 mg/l and 14.16 mg/l respectively, while the nitrate and nitrite content of demineral water after boiling process contained 37.11 mg/l and 11.48 mg/l respectively, since nitrite and nitrate migrated from vegetables in to the water during boiling [11;19]. So that it is not recommended to use the broth or water resulted from boiling process of vegetable such as soup for baby foods since baby still is sensitive towards nitrite and nitrate [11].

Conclusion

The level of nitrate is higher than nitrite present in fresh celery, where as nitrite level was much higher than usually present in most vegetables. The boiling time and water used affecting the nitrate and nitrite content in celery. The longer the boiling the lower the nitrate and nitrite content in celery. The effect of demineral water is more influential to lower the nitrate and nitrite content of celery. The nitrate and nitrite migrated from celery into water used during boiling indicated by increasing and presence of nitrate and nitrate content in water after boiling process.

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