The Effect of Boiling Process using Various Types of Containers on Nitrate, Nitrite and Vitamin C Contents in Vegetables

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Keywords: Vegetables, nitrite, nitrate, Vitamin C, boiling container, Spectrophotometry, 2,6-dichlorophenolindophenol.

Abstract: The aimed of this study was to determine the effect of boiling using various types of containers on nitrite, nitrate, and vitamin C levels in vegetables. The samples analyzed were mustard, bok coy, spinach, and lettuce derived from Traditional Markets. The treatment process uses a variety of containers are stainless steel, stoneware, aluminum, and a pitcher. Boiling is done for 5 minutes. Nitrite was derivatized with sulfanilic acid and N-(1-naphthyl) ethylenediamine dihydrochloride in acetic acid solution, then absorbance measured by spectrophotometer at 540 nm. Nitrate reduced to nitrite by adding zincum powder in acidic condition, then determined as nitrite. Vitamin C was titrated with 2,6-dichlorophenol indophenols. The results of this study, on the boiling process using containers, showed that during the boiling process nitrite, nitrate, and vitamin C levels decreased. The most influential type of boiling container is boiling with aluminum containers. During the boiling process using containers can reduce the level of vitamin C. Boiling process using containers should use stainless steel container. The use of stainless steel containers has decreased nitrate levels greater than other containers.

1 INTRODUCTION

Vegetables are an important role in human nutrition since they are an outstanding source of vitamins, minerals, and biologically active compounds. Despite the nutritional benefit of eating vegetables, they also contain substances that adversely affect human health such as nitrites and nitrates (Prasad and Chetty, 2007).

Vegetables are a major source of nitrite and nitrate intake from food. Nitrite and nitrate are also used as preservatives and coloring agents in processed meats. Nitrate and nitrite contents in vegetables vary widely from 1 to 10.000 mg/kg, and this is affected by many factors, including environmental factors such as storage condition, processing procedure, temperature and agricultural practices (Prasad and Chetty, 2007,).

Nitrate can be reduced into nitrite by enzyme nitrate reductase and other reducing agents, including vitamin C, which is also contained in vegetables. Nitrite may react with alkylamines to form carcinogenic nitrosamines[10-11]. Therefore, the intake permitted (Acceptable Daily Intake = ADI) by the Food and Agriculture Organization of the United Nations/World Health Organization is 220 mg of nitrate and 8 mg of nitrate per day for adults weighing an average of 60 kg (Amr and Hadidi, 2014; Hill, 1996; Keshavarz, et al., 2015).

Several factors may affect the accumulation of nitrates in vegetables including cooking process, type of utensil, fertilization, and storage condition[4]. This cooking process may change vegetable composition either in a positive or negative way (Leszczynska, et al., 2009). Mitigation of nitrite and nitrate intake may result from processing e.g., washing, peeling and boiling. The vegetable is usually processed by boiling in a utensil made of a different metal component such as aluminum, zinc, iron, chromium and in combination with different reactivity in water affected by heat on the cooking process. The previous study reported that cooking using metal component utensils may bring about the reduction of nitrate to nitrite (Hill, 1996). The aim of this study was to investigate the effect of boiling using various types of containers on nitrite, nitrate, and vitamin C levels in some vegetables in the Medan City.

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2 MATERIALS AND METHOD

2.1 Materials

Chemicals used were analysis grade products from Merck KGaA (Germany): N-(1-naphthyl) ethylenediamine dihydrochloride (NED), sodium nitrite, sulfanilic acid, glacial acetic acid, hydrochloric acid, antipyrine, ferrous sulfate, zinc powder, sodium nitrite, ascorbic acid, metaphosphoric acid, and 2.6-dichlorophenolindophenol.

2.2 Samples Preparation

The vegetables analyzed in this research were sweet mustard (*Brassica rapa chinensis)*, bok choy (*Brassica rapa* L.), spinach (*Amaranthus tricolor* L), and lettuce (*Lactuca sativa* L.). The vegetables obtained from Lokal Market in Pasar V Padang Bulan, Medan. The Vegetables were cleaned, and then weighed 10 g and then cut into small pieces (± 4 cm). The vegetables were placed in an aluminum pan, periuk, stainless steel and pitchers pan containing 500 ml of boiling water. The boiling process was carried out for 5 minutes. This process was assumed to be similar to normal households cooking. Then nitrate, nitrite, and vitamin C contents in samples were analyzed.

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2.3 Determination of Absorbance Curve of Nitrite Standard Solution

Four (4) ml of a standard solution of nitrite (C=10.0 μg/ml) was transferred into 50 ml volumetric flask, added 2.5 ml sulfanilic acid solution and shaken, after 5 min 2.5 ml NED reagent was added and made to volume with distilled water and homogenized (C=0.8 μg/ml). Absorbance was measured at a wavelength of 400-800 nm. Then, absorbance and wavelength were plotted to construct the absorbance curve. The wavelength of maximum absorbance was determined from the absorbance curve (Cintya, et al., 2018).

2.4 Absorbance Stability of Derivatized Nitrite to Determine Working Time

Four (4) ml of a standard solution of nitrite (C=10.0 μg/ml) was transferred into a volumetric flask of 50 ml, to which 2.5 ml of sulfanilic acid and stirred. After 5 min, 2.5 ml NED reagent was added and distilled water was added to make 50 ml. Absorbance was measured at the wavelength of maximum absorbance obtained from the absorbance curve (540 nm), and stability of absorbance was determined by observing absorbance at every minute for 1 hr. The absorbance was found to be relatively stable within 6 min in 7-12 min (Cintya, et al., 2018).

2.5 Determination of Calibration Curve

Standard solution of nitrite (C=10.0 μg/ml) of different volume (0.5,1,2,3,4 dan 5 ml) were transferred into separated volumetric flasks of 25 ml, then 2.5 ml sulfanilic acid reagent added and stirred to homogenize. After 5 min, 2.5 ml NED reagent was added, then distilled water was added to make a volume of 25 ml and homogenized. The series of concentration of prepared solutions were of 0.1 μg/ml, 0.2 μg/ml, 0.4 μg/ml, 0.8 μg/ml, 1.0 μg/ml. The absorbance of each solution was measured at a wavelength of 540 nm within 7 min. The calibration curve was made by plotting absorbance versus concentration of each solution. From the graph obtained, then linearity of the regression equation and correlation coefficient were calculated (Y=aX+b) (Cintya, et al., 2018).

2.6 Identification of Nitrite and Nitrate in Vegetables

About 10 g ground sample using blender as transferred into a glass beaker. Distilled water was added about 150 ml, heated in a water bath (80ºC) and shaken for 5 minutes then cooled and filtered. The supernatant was transferred into a test tube, then 2.5 ml sulfanilic acid reagent was added and stirred. After 5 minutes, 2.5 ml reagent NED was added. Nitrite was identified using sulfanilic acid and NED solution, and the appearance of a violet color indicated the presence of nitrite. Nitrate was identified by adding several drops ferrous sulfate solution and then slowly adding a few drops of concentrated sulfuric acid. The formation of a chocolate ring indicates the presence of nitrate (Cintya, et al., 2018).

2.7 Quantification of Nitrite and Nitrate in Vegetables

2.7.1 Nitrite

Determination of nitrite was carried out with the procedure previously describe (Cintya, et al., 2018). Around ten (10) gram, grounded sample transferred into 250 ml beaker glass to which hot distilled water (± 80ºC) was added about 150 ml. This mixture was homogenized by stirring and heated on a water bath for 15 minutes while stirring. Allowed to cool and then transferred quantitatively into 250 ml volumetric flask, distilled water added to the volume, then filtered. 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 added to make 50 ml, and then homogenized. Absorbance was measured at a wavelength of 540 nm after a period of 7 to 12 minutes time.

2.7.2 Nitrate

Around ten (10) gram, grounded sample transferred into 250 ml beaker glass to which hot distilled water (± 80ºC) was added about 150 ml. This mixture was homogenized by stirring and heated on a water bath for 15 minutes while stirring. Allowed to cool and then transferred quantitatively into 250 ml volumetric flask, distilled water added to the volume, then filtered. Ten (10 ml) of filtrate transferred into a volumetric flask of 50 ml, then 0.1 g Zn powder and 1 ml HCl 1 N added and allowed to stand for 10 minutes to reduce nitrate to nitrite, then 2.5 ml sulfanilic acid reagent was added and stirred. After 5 minutes, 2.5 ml reagent NED was added, then distilled water added to make 50 ml, and then homogenized. Absorbance was measured at a wavelength of 540 nm after a period of 7 to 12 minutes time. Nitrite concentration from the reduction of nitrate into nitrite was calculated:

$$a = \mathrm{b} - c$$

Note:

a: Nitrite concentration from nitrate reduction
b: Total nitrite content
c: Initial nitrite concentration in samples
Nitrate content was calculated:

$$\text{Nitrite concentration from nitrate reduction} = \text{Concentration of nitrate} \; x \; \frac{Molecule\ Weigt\ nitrate}{Molecule\ Weigt\ nitrite}$$

2.8 Identification of Vitamin C in Vegetables

Ten (10) gram grounded sample using a blender. About 0.5 ml of the sample solution in a test tube was neutralized to a pH of 6-8 with NH_4OH 1 N, three drops of 3% $FeCl_3$ was added – a purple color indicates the presence of vitamin C (Ditjen POM, 1995).

2.9 Analysis of Vitamin C in Vegetables

Ten (10) gram grounded sample using blender was transferred into 100 ml volumetric flask, then acetic metaphosphoric acid 3% added to make 100 ml, then homogenized and filtered. Two (2) ml of filtrate was transferred into an Erlenmeyer, and then added 5 ml of acetic metaphosphoric acid, then titrated with 2.6-dichlorophenol indophenol solution 0.025% until pink steadily (Ditjen POM, 1995). The levels of vitamin C was calculated.

$$\text{Vitamin C (mg/g)} = \frac{(Vt - Vb) \times Equivalence \times VL}{Vp \times Bs}$$

Vb = The volume of blank (ml); VL = The volume of volumetric flask (100 ml); Vp = The volume of pipetted sample solution(ml); Bs = Sample weight (g)

2.10 Statistical Analysis

SPPS for windows version 23.0 was used for statistical analysis. All experiments carried out in six replicates. The data were analyzed using ANOVA. The differences between the mean values were separated using post hoc tests at a significant level of $P < 0.05$.

3 RESULTS AND DISCUSSION

3.1 Nitrite and Nitrate Identification in Samples

It is found that samples contain nitrite indicated by the appearance of violet color to prove that all samples contained nitrate. The reaction with antipyrine in dilute hydrochloric resulted in the formation of green color to prove the presence of nitrite. Nitrate in samples was identified using ferrous sulfate and concentrated sulfuric acid produced brown ring (Cintya, et al., 2018).

3.2 Identification of Vitamin C in Samples

Identification of vitamin C was done using $FeCl_3$ 3% and ammonia silver reagents. Vitamin C identification using $FeCl_3$ 3% reagent indicated by the appearance of violet color to prove that all samples contained vitamin C. Identification with ammonia silver reagent resulted in the brown ring also indicated the presence of Vitamin C in samples (Ditjen POM, 1995).

3.3 The Wave-Length of Maximum Absorption of Nitrite Derivative

Maximum absorption was determined from the absorption curve of nitrite derivative solution (10 µg/ml). Nitrite derivative solution at a concentration of 10 µg/ml within the range of wavelength from 400 nm through 800 nm and the result is presented in Figure 1.

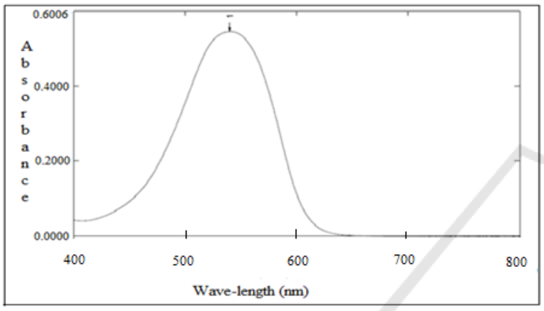

Figure 1: Absorbance Curve of Nitrite Derivative.

From Figure 1 it is found that maximum absorption was 540 nm which is similar to that the value previously reported[1]. The wavelength of 540 nm was then used to determine the working time for analysis of nitrite and nitrate in samples (Cintya, et al., 2018).

3.4 Working Time for Measurement

Working time for measurement for nitrite and nitrate analysis was determined to know the period of time within which the absorbance of solution still remains stable. The absorbance of nitrite derivative with *Griess* reagent presented in Figure 2.

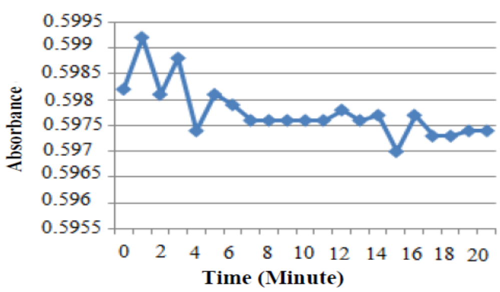

Figure 2: Absorbance of Nitrite Derivative with Time.

From Figure 2 can be seen that the absorbance was stable within minute 7 to minute 12 which is called working time. This working time was then applied in the analysis procedure for nitrite and nitrate in samples (Cintya, et al., 2018).

3.5 Calibration Curve of Nitrite Derivative

Calibration Curve made by plotting absorbance versus concentration of each solution, then the linearity of the regression equation was determined. The calibration curve presented in Figure 3.

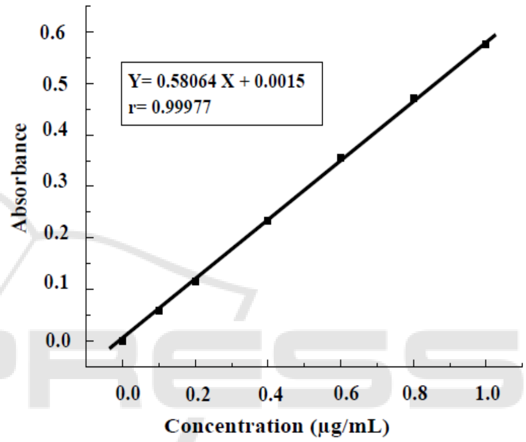

Figure 3: Calibration Curve of Nitrite Derivative.

Regression equation obtained is Y= 0.58064X + 0.0015 with coefficient correlation (r) of 0.99977 (where r > 0.999). Quantification of nitrite and nitrate was calculated using this regression equation. From Figure 3 it is found that the correlation coefficient was calculated (r=0.999) which indicates that the correlation between concentration and absorbance is high. Calibration curve indicated linearity between concentration and absorbance (Cintya, et al., 2018).

3.6 The Effect of Boiling Process using Various Types of Containers on Nitrate, Nitrite and Vitamin C Contents in Vegetables

The effect of the boiling process using various types of containers on nitrate, nitrite and vitamin c contents in vegetables can be seen in Table 1.

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Table 1: The Effect of Boiling Process Using Various Types of Containers on Nitrate, Nitrite and Vitamin C Contents in Vegetables.

Vegetables	**Type of Utensil for Boiling Process**	**Nitrite Levels (mg/kg) ± SD**	**Nitrate Levels (mg/kg) ± SD**	**Vitamin C Levels (mg/100g)**
Mustard	Beaker glass	15.23 ± 0.18*	22.46 ±0.24*	99.57 ± 0.39*
	Stainless steel	12.29 ± 0.07*	15.96 ± 2.45*	49.16 ± 1.04
	Stoneware	12.51 ± 0.06*	17.60 ± 0.20*	48.62 ± 0.75
	Aluminium	13.39 ± 0.09*	20.52 ± 0.10	45.99 ± 1.09
	Pitcher	12.80 ± 0.12*	20.09 ± 0.14	46.39 ± 0.65
Bokcoy	Beaker glass	12.57 ± 0.22*	6.55 ± 0.28*	90.73 ± 0.19*
	Stainless steel	12.37 ± 0.07*	5.55 ± 0.16*	31.10 ± 0.99
	Stoneware	12.29 ± 0.08*	5.74 ± 0.24*	32.24 ± 1.60
	Aluminium	12.43 ± 0.07	5.95 ± 0.11	25.48 ± 0.59*
	Pitcher	12.41 ± 0.08	5.99 ± 0.19	28.90 ± 0.82*
Spinach	Beaker glass	20.26 ± 0.09*	90.60 ± 0.35*	87.56 ± 0.67*
	Stainless steel	16.07 ± 0.02*	37.36 ± 0.17*	46.42 ± 0.85
	Stoneware	17.35 ± 0.03*	36.01 ± 0.10*	45.17 ± 0.84
	Aluminium	19.54 ± 0.13*	40.20 ± 0.20*	33.91 ± 0.51*
	Pitcher	17.71 ± 0.15*	37.56 ± 0.37*	41.82 ± 1.32*
Lettuce	Beaker glass	18.77 ± 0.01*	32.68 ± 0.15*	39.41 ± 0.88*
	Stainless steel	14.77 ± 0.12*	25.57 ± 0.15*	22.60 ± 0.82*
	Stoneware	15.13 ± 0.05*	28.14 ± 0.14*	19.88 ± 0.84*
	Aluminium	16.64 ± 0,11*	31.65 ± 0.19*	18.63 ± 0.85
	Pitcher	15.56 ± 0.11*	29.01 ± 0.08*	18.64 ± 0.99

Note: Values is the mean of six replicates; *. The mean difference is significant at the 0.05 level).

In Table 1 it can be seen that in fresh vegetables that nitrite, nitrate, and vitamin C levels are higher than the processing process using boiling containers. This is because boiling can reduce nitrate levels because nitrates have a tendency to dissolve easily in cooking water (Huarte-Mendicoa, et al., 1997). During boiling, vegetables are directly related to the heat produced by boiling water, so that the cell walls and plasma membranes are rapidly damaged. Thus, boiling water enters the cell wall and vacuole which then dissolves the phenol compound into the processing liquid (Lund, 1977).

Changes in the levels of nitrite, nitrate, and vitamin C are different by using different types of containers. During the boiling process using a container, there is a decrease in nitrite and nitrate levels and vitamin C levels are seen from various vegetables examined. In this study it was found that in aluminum containers there was a change in nitrate to nitrite which was not significant where nitrate levels decreased less but nitrite levels were increased which mean the process of contact with aluminum containers is not significant that changes in nitrate to nitrite occur less, when compared to other types of containers.

The results of this study are different from previous studies conducted by Silalahi, et al., 2017 where the greatest reduction in nitrate levels found in aluminum containers is indicated by the high influence of the nitrate reduction process to nitrite as evidenced by the longer boiling time increasing nitrite levels. Where in this study is expected that aluminum nitrate levels in more decreases, whereas nitrite levels are slightly lower than other containers? This is likely to occur because there are other factors that influence it, such as a short boiling time so that contact with the container used is not very visible so that changes in nitrate to nitrite also do not occur.

The equipment used when cooking can also affect the levels of nitrate and nitrite because nitrate was reduced to nitrite due to contact with metals originating from containers used especially aluminum containers. Therefore, the constituent metal used can affect the levels of nitrate and nitrite. When connected with electrochemical series that the constituent components of stainless steel and pots, which generally consist of Cr, Ni, Fe, and Cu, in this case, the position of the metal in the right position which means it is less reactive and more difficult to release electrons. So that it is difficult to reduce nitrate to nitrite. Whereas in aluminum containers and jugs which generally consist of Al and other metal mixtures such as Mg, Mn, Zn, and others. The position of the metal in the left position which means it is more reactive and readily releases electrons, so that can reduce nitrate to nitrite, this can occur because there is an electron donor in the form of hydrogen which reacts between the metals during heating (Dogra, 1990).

Vitamin C levels decrease after the boiling process, this is because the preparation of vegetables with cooking will damage vitamin C, so it is recommended when cooking vegetables should be half-cooked to avoid denaturation of plant tissue or cell disruption and separation of some phenolic compounds from cellular structures that contain vitamin C and bioavailability increased so as to prevent the formation of cancer risk. The greatest decrease in vitamin C levels after the boiling process is in aluminum containers compared to other types of containers (Raczuk, et al., 2014; Silalahi, 2005; Vahed, et al., 2015; Walters, 1996).

4 CONCLUSIONS

The boiling process using a container indicates that decreased levels of nitrite and nitrate in mustard, bok coy, spinach, and lettuce. A greater decrease in the levels of nitrite and nitrate is found in stainless steel containers. During the boiling process using containers can reduce vitamin C levels, the biggest decrease is found in aluminum containers

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