

The Effect of Boiling Duration on The Antioxidant Activity of Green Mustard (*Brassica rapa L.*)

Landung Wahyu Prasetya¹, Sri Wahyuni², Nur Mahmudah³, Sa'idatul Fithriya⁴

¹⁾ Student of Faculty Medicine, Muhammadiyah University of Surakarta, Surakarta City, East Java Province, Indonesian

^{2,3,4)} Lecturer of Faculty Medicine, Muhammadiyah University of Surakarta, Surakarta City, East Java Province, Indonesian

Email: landungwahyu2002@gmail.com

ABSTRACT

Background: A study stated that one of the contents of green mustard is phenolic which acts as an antioxidant. Antioxidant activity is influenced by the processing method, especially boiling. The duration of boiling can cause damage to antioxidant compounds. **Outcome:** This study was to determine the effect of the duration of boiling green mustard on the antioxidant activity contained in it. **Method:** This study used a laboratory experimental method with a post-test only controlled group using the 2,2-difenil-1-pikrilhidrazil (DPPH) method to measure the antioxidant activity of boiled green mustard. **Results:** The results of testing on fresh green mustard, green mustard boiled for 2-4 minutes showed an increase in IC50 value from $4,414.03E + 48$ ppm to $5.3805E + 138$ ppm. Green mustard boiled for 6 minutes experienced a decrease in IC50 value from $5.3805E + 138$ ppm to $2,505.23E + 45$ ppm. The IC50 value of green mustard boiled for 8 minutes increased again to $5,051.02E + 48$ ppm. Boiled mustard greens for 10 minutes had a significantly decreased IC50 value to 0.0469 ppm. **Conclusion:** The effect of the duration of boiling green mustard greens (*Brassica rapa L.*) on the antioxidant effects contained therein. The duration of boiling green mustard greens (*Brassica rapa L.*) which has good antioxidant activity in green mustard greens boiled for 10 minutes.

Keyword: Green Mustard, Phenolic, Antioxidant, Post Test Only Controlled Group

Introduction

Degenerative diseases are chronic diseases caused by decreased organ and tissue function (Kemenkes RI, 2022). Based on world epidemiological data, it was found that 17 million people died from degenerative diseases in countries with low-medium national incomes (Direnzo *et al.*, 2021). The prevalence of degenerative diseases that can cause death is 80% and is expected to continue to increase almost twofold in the next few years (WHO, 2023). Risk factors that play a role in accelerating the occurrence of degenerative diseases are oxidative stress. The balance between the formation of free radicals and the ability of cells to clean them, so that it can induce chronic diseases, accelerate body aging and acute pathologies such as stroke (Pizzino *et al.*, 2017).

Antioxidants play an important role in protecting body cells from oxidative damage that can cause several types of diseases including cancer, heart disease and premature aging (Bjelakovic *et al.*, 2014). Some literature explains that giving antioxidants at overdose or low doses with an unhealthy lifestyle can initiate the risk of cancer, cardiovascular disease and increase the risk of death. There are 2 types of antioxidants, namely endogenous antioxidants and exogenous antioxidants. Antioxidants in the body in the form of enzymes play a role in preventing the production of ROS, also called endogenous antioxidants. These antioxidants consist of superoxide dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPX), and Hydropersulfide (Nursini, Venti and Wita, 2020). Meanwhile, exogenous antioxidants are antioxidants obtained from outside such as food, plants and others (Pruteanu *et al.*, 2023). Antioxidants derived from plants have neuroprotective functions including polyphenols, tocopherols, carotenoids, ascorbic acid, and polysaccharides and peptides (Cui *et al.*, 2020; Michalak, 2022). Green mustard greens or choy sum (*Brassica rapa L.*) is a leafy vegetable grown in Asia, a member of the genus *Brassica* in the family Brassicaceae (*Brassica rapa var. parachinensis* or *Brassica chinensis var. parachinensis*) (Wang *et al.*, 2022; Chung *et al.*, 2013).

Green mustard greens are rich in bioactive compounds and secondary metabolites such as glucosinolates, phenolics, carotenoids, flavonoids, anthocyanins, vitamin C and vitamin E (Zou *et al.*, 2021). These compounds have antioxidant properties that protect the body from inflammation or heart disease. Antioxidants can react with DPPH (2,2-diphenyl-1-picrylhydrazine) by adding hydrogen atoms or electrons to the radical center due to the reaction with antioxidants (Flieger, 2020). The absorption of antioxidants in green mustard greens is influenced by the boiling process (Multescu *et al.*, 2019). Boiling is a form of moist heat sterilization that uses water to boil or steam which depends on the boiling point of a liquid with the surrounding air pressure (Lapenna, 2018). Lower air pressure produces a low boiling point, conversely if the air pressure is high then the boiling point is higher (Tugba *et al.*, 2022).

Antioxidant activity can be affected by processing methods, especially boiling. Boiling can affect the content of bioactive compounds in vegetables. Boiling time can damage antioxidant compounds, thereby reducing the antioxidant activity that

vegetables can provide to the body. Boiling is influenced by air pressure and boiling time so that oxidation, hydrolysis and release of antioxidant peptides occur. Green mustard will experience antioxidant degradation causing reduced antioxidant content (Ghanad *et al.*, 2020).

Methodology

This study used a laboratory experimental research type with the Post-test only controlled group design method using the 2,2-diphenyl 1-picrylhydrazyl (DPPH) method used to measure the antioxidant activity of boiled green mustard greens. This study was conducted at the Pharmacology Laboratory of the Faculty of Medicine, Muhammadiyah University of Surakarta. Determination of plants used as samples in this study was carried out at the Laboratory of the Center for Research and Development of Medicinal Plants and Traditional Medicines, Tawangmangu with the number TL.02.04/D.XI.06/133.073/2024. Sampling was done using the purposive sampling method. The samples used were fresh green mustard greens and variations in boiling time homogenized with methanol into 36 groups consisting of concentrations of 1000 ppm, 500 ppm, 250 ppm, 125 ppm, 62.5 ppm, 31.25 ppm. The positive control used was boiled and unboiled Vitamin C with the same concentration and variation in boiling time, namely 2, 4, 6, 8, and 10 minutes. Preparation of DPPH solution by dissolving DPPH powder that has been weighed and homogenized with PA methanol. Calculation of inhibition percentage using the formula Percent Inhibition = $(\text{Control absorbance} - \text{Sample absorbance}) / (\text{Control absorbance}) \times 100\%$.

There are two hypotheses in this study: 1) The duration of boiling green mustard will affect its antioxidant activity. 2) The duration of boiling green mustard does not affect its antioxidant activity.

Result and Discussion

The results of the DPPH test consist of determining the maximum DPPH wavelength, determining the working time, and determining the antioxidant activity. The results of the wavelength measurement at 400-600 nm obtained maximum absorption at 517 nm so that it can be used to see the absorbance of the sample. The results of the working time measurement were obtained at the 30th minute, the absorbance value of the green mustard sample and the positive control of vitamin C began to show a stable value. Measurement of antioxidant activity was carried out by replication 3 times using a UV-Vis spectrophotometer. To see the antioxidant activity in the tested samples using the IC₅₀ value parameter. The following are the results obtained from the research:

Table 1. Inhibition Percentage Value of Green Mustard Greens

Sample	Concentration	Average Percentage Inhibition
Fresh Green Mustard Greens	1000 ppm	-120,814
	500 ppm	-123,994
	250 ppm	-128,225
	125 ppm	-125,389
	62,5 ppm	-128,552
	31,25 ppm	-125,046
Boil Green Mustard Greens for 2 Minutes	1000 ppm	-120,623
	500 ppm	-125,589
	250 ppm	-127,947
	125 ppm	-126,894
	62,5 ppm	-127,649
	31,25 ppm	-125,727
Boil Green Mustard Greens for 4 Minutes	1000 ppm	-120,321
	500 ppm	-126,950
	250 ppm	-126,147
	125 ppm	-125,550
	62,5 ppm	-127,775
	31,25 ppm	-128,555
Boil Green Mustard Greens for 6 Minutes	1000 ppm	-122,469
	500 ppm	-125,951
	250 ppm	-124,805
	125 ppm	-129,386
	62,5 ppm	-126,225
	31,25 ppm	-125,011
Boil Green Mustard Greens for 8 Minutes	1000 ppm	-121,061
	500 ppm	-126,711
	250 ppm	-127,216
	125 ppm	-128,204
	62,5 ppm	-125,792
	31,25 ppm	-125,425
Boil Green Mustard Greens for 10 Minutes	1000 ppm	-119,135
	500 ppm	-84,568
	250 ppm	-85,366
	125 ppm	-83,747
	62,5 ppm	-90,510
	31,25 ppm	-81,353

Table 2. Inhibition Percentage Value of Vit C

Sample	Concentration	Average Percentage Inhibition
Vit C without Boiling	1000 ppm	95,157
	500 ppm	95,470
	250 ppm	91,211
	125 ppm	73,226
	62,5 ppm	11,983
	31,25 ppm	-9,541
Vit C Boil for 2 Minutes	1000 ppm	95,072
	500 ppm	95,302
	250 ppm	95,051
	125 ppm	93,047
	62,5 ppm	78,618
	31,25 ppm	13,886
Vit C Boil for 4 Minutes	1000 ppm	95,302
	500 ppm	95,114
	250 ppm	93,443
	125 ppm	70,537
	62,5 ppm	9,417
	31,25 ppm	-15,014
Vit C Boil for 6 Minutes	1000 ppm	95,114
	500 ppm	95,218
	250 ppm	92,044
	125 ppm	69,242
	62,5 ppm	27,125
	31,25 ppm	-5,638
Vit C Boil for 8 Minutes	1000 ppm	95,385
	500 ppm	95,176
	250 ppm	92,483
	125 ppm	65,985
	62,5 ppm	9,626
	31,25 ppm	-14,074
Vit C Boil for 10 Minutes	1000 ppm	95,427
	500 ppm	94,801
	250 ppm	95,364
	125 ppm	58,843
	62,5 ppm	6,160
	31,25 ppm	-15,055

Table 3. IC₅₀ Value Result of Green Mustard Greens

Sample	Replication	Regression Equation	IC ₅₀ Value	Average IC ₅₀ Value
Fresh Green Mustard Greens	1	$Y = 2,3957x - 140,46$	3,36315E+34	4.414,03E+48
	2	$Y = 1,6235x - 133,63$	13.242,1E+49	
	3	$Y = 3,7519x - 145,32$	4,06366E+22	
Boil Green Mustard Greens for 2 Minutes	1	$Y = 3,0611x - 142,07$	1,77823E+27	3.649,5E+57
	2	$Y = 1,3671x - 132,7$	1.094,85E+58	
	3	$Y = 4,7352x - 154,79$	6,06119E+18	
Boil Green Mustard Greens for 4 Minutes	1	$Y = 2,0747x - 135,68$	7,38199E-38	5,3805E+138
	2	$Y = 3,9509x - 149,53$	8,56882E+21	
	3	$Y = 0,4169x - 126,83$	1.614,2E+184	
Boil Green Mustard Greens for 6 Minutes	1	$Y = 2,5477x - 139,31$	1,86547E+32	2.505,23+45
	2	$Y = 17645x - 136,39$	7,5157+45	
	3	$Y = 4,1734x - 151,05$	8,35156E+20	
Boil Green Mustard Greens for 8 Minutes	1	$Y = 2,6939x - 139,62$	371026E+30	5.051,02+48
	2	$Y = 5,1591x - 157,74$	3,07335E+17	
	3	$Y = 1,6402x - 135,74$	15.153,1+49	
Boil Green Mustard Greens for 10 Minutes	1	$Y = -12,14x - 22,45$	0,0025597	0,0469552
	2	$Y = -16,401x - 4,0833$	0,0608327	
	3	$Y = -17,063x - 17,3559$	0,0774734	

Table 4. IC₅₀ Value Result of Vit C

Sample	Replication	Regression Equation	IC ₅₀ Value	Average IC ₅₀ Value
Vit C without Boiling	1	$Y = 10,102x - 29,49$	7,61630262	7,62
	2	$Y = 10,102x - 29,49$	7,61630262	
	3	$Y = 10,102x - 29,49$	7,61630262	
Vit C Boil for 2 Minutes	1	$Y = 0,9038x - 89,309$	1,29187	2,64236
	2	$Y = 0,9038x - 89,309$	1,29187	
	3	$Y = 0,9309x - 89,166$	5,34336	
Vit C Boil for 4 Minutes	1	$Y = 10,954x - 24,323$	10,42362	10,44
	2	$Y = 10,954x - 24,323$	10,42362	
	3	$Y = 10,972x - 24,217$	10,48442	
Vit C Boil for 6 Minutes	1	$Y = 11,649x - 19,535$	13,67057	13,68
	2	$Y = 11,658x - 19,498$	13,68637	
	3	$Y = 11,658x - 19,498$	13,68637	
Vit C Boil for 8 Minutes	1	$Y = 13,104x - 10,355$	20,60249	20,63
	2	$Y = 13,104x - 10,355$	20,60249	
	3	$Y = 13,132x - 10,211$	20,69571	
Vit C Boil for 10 Minutes	1	$Y = 15,752x - 6,3273$	35,72590	35,73
	2	$Y = 15,752x - 6,3273$	35,72590	
	3	$Y = 15,752x - 6,3273$	35,72590	

From the research data, the IC₅₀ value of fresh green mustard greens was 4,414.03E+48 ppm. The IC₅₀ value of 2-minute boiled mustard greens was 3,649.5E+57 ppm. The 4-minute boiled mustard greens had an IC₅₀ value of 5.3805E+138 ppm. Then the IC₅₀ value of 6-minute boiled mustard greens was 2,505.23E+45 ppm. The IC₅₀ value of 8-minute boiled mustard greens was

5,051.02E+48 ppm. The IC50 value of 10-minute boiled mustard greens was 0.649 ppm. IC50 value data of green mustard, antioxidant activity of green mustard is classified as very weak because it is greater than 200 ppm except for green mustard boiled for 10 minutes. The IC50 value of the positive control of vitamin C without boiling is 7.62 ppm. Then the IC50 value of vitamin C boiled for 2 minutes is 2.64236 ppm. In vitamin C boiled for 4 minutes, it has an IC50 value of 10.44 ppm. The IC50 value of vitamin C boiled for 6 minutes is 13.68 ppm. The IC50 value of vitamin C boiled for 8 minutes is 20.63 ppm. Vitamin C boiled for 10 minutes has an IC50 value of 35.73 ppm. Positive control of vitamin C has very strong antioxidant activity because its IC50 value is less than 50 ppm.

The test results on fresh green mustard greens, boiled green mustard greens for 2 and 4 minutes showed an increase in IC50 value from 4,414.03E+48 ppm to 5.3805E+138 ppm. Then boiled green mustard greens for 6 minutes experienced a decrease in IC50 value from 5.3805E+138 ppm to 2,505.23E+45 ppm. The IC50 value of boiled green mustard greens for 8 minutes increased again to 5,051.02E+48 ppm. While boiled mustard greens for 10 minutes had a significant decrease in IC50 value to 0.0469 ppm. The antioxidant activity of fresh green mustard greens is classified as very weak because the IC50 value is more than 200 ppm (4,414.03E+48 ppm). This study obtained the strongest antioxidant activity of green mustard greens in green mustard greens with boiling for 10 minutes (0.0469 ppm). The results are opposite to the positive control. In the positive control test, vitamin C has a very strong antioxidant activity in vitamin C without boiling.

As mentioned by Ghanad *et al.* (2020), boiling is one of the processing procedures that might impact antioxidant activity. Vegetables' bioactive component composition may change when they are boiled. The antioxidant activity that vegetables may offer the body is diminished by boiling time, which can harm antioxidant molecules. Air pressure and boiling time affect boiling, causing oxidation, hydrolysis, and the release of antioxidant peptides. Green mustard's antioxidant content will decrease as a result of antioxidant degradation.

This study is in line with the study conducted by Tugba *et al.* (2022) who obtained results on celery, leeks, and spinach after cooking. Boiling time affects the total phenolic content. The antioxidant activity of leek samples was found to increase when boiled for 15 minutes compared to boiling for 5 minutes. The increase in phenolic content values observed in their study partly contradicts the claim that cooked vegetables have lower nutritional value than raw ones. Increased antioxidant activity during cooking may be due to changes in the matrix and an increase in compounds that can be converted into new antioxidants. Boiling is one of the cooking methods that can affect the nutritional content of food. Boiling in some vegetables has been found to increase antioxidant activity. Boiling can have varying effects on antioxidant activity depending on the type of phenolic compounds each vegetable has.

In a study by Sud *et al.* (2022) it was observed that boiling of potatoes and sweet potatoes led to a significant increase in free phenolic acids, except 3-O-caffeoylquinic acid and 3,4-O-dicaffeoylquinic acid, which decreased during the

process. They did not mention other specific types of antioxidants that increased due to boiling (Shivani., 2022). Therefore, boiling has been shown to increase the levels of various antioxidants, including phenolic compounds and carotenoids (Sanda et al., 2019).

Conclusion

The duration of boiling green mustard greens (*Brassica rapa L.*) affects its antioxidant activity and the duration of boiling green mustard greens (*Brassica rapa L.*) which has good antioxidant activity is 10 minutes of boiled green mustard greens. Environmental factors such as variations in soil quality or climate may affect the antioxidant activity of mustard greens. Other cooking parameters such as temperature and water volume were not studied in depth, and these factors may affect antioxidant activity. The results may be specific to mustard greens (*Brassica rapa L.*) and may not be applicable to other vegetables with different bioactive compound compositions.

Declaration of Competing Interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Reference

- Bjelakovic, G., Nikolova, D. and Glud, C. (2014) 'Antioxidant supplements and mortality', *Current Opinion in Clinical Nutrition and Metabolic Care*, pp. 40–44. Available at: <https://doi.org/10.1097/MCO.0000000000000009>.
- Chung, W.Q., Ahmad, S. H., Zamri, M. Z., and Rosenani, A. B. (2013) 'Nitrate and nitrite contents and postharvest quality of choy sum (*Brassica rapa chinensis* Group) during storage', in *Acta Horticulturae*. International Society for Horticultural Science, pp. 315–320. Available at: <https://doi.org/10.17660/ActaHortic.2013.1012.39>.
- Cui, X., Lin, Q. and Liang, Y. (2020) 'Plant-Derived Antioxidants Protect the Nervous System From Aging by Inhibiting Oxidative Stress', *Frontiers in Aging Neuroscience*. Frontiers Media S.A. Available at: <https://doi.org/10.3389/fnagi.2020.00209>.
- Di Renzo, Laura., Gualtieri, Paola. and De Lorenzo, Antonino. (2021) 'Diet, nutrition and chronic degenerative diseases', *Nutrients*. MDPI AG. Available at: <https://doi.org/10.3390/nu13041372>.
- Flieger, J. and Flieger, M. (2020) 'The [DPPH•/DPPH-H]-HPLC-DAD Method on Tracking the Antioxidant Activity of Pure Antioxidants and Goutweed (*Aegopodium podagraria L.*) Hydroalcoholic Extracts', *Molecules*, 25(24). Available at: <https://doi.org/10.3390/MOLECULES25246005>.
- Ghanad, Mona., Nulit, Rosimah., Go, Rusea., and Seok, Christina Yong. (2020) *Comparative Study on Antioxidant Activity of Brassica rapa var. parachinensis Leaves and Seeds*, *Advances in Nutrition and Food science*. Available at: www.kosmospublishers.com.
- Kemenkes RI. (2022). Penyakit Degeneratif. Diambil dari: https://yankes.kemkes.go.id/view_artikel/1714/penyakit-degeneratif

Lapenna, P.E. (2018) 'Characterization of pseudo-boiling in a transcritical nitrogen jet', *Physics of Fluids*, 30(7). Available at: <https://doi.org/10.1063/1.5038674>.

Michalak, M. (2022) 'Plant-Derived Antioxidants: Significance in Skin Health and the Ageing Process', *International Journal of Molecular Sciences*. MDPI. Available at: <https://doi.org/10.3390/ijms23020585>.

Mulțescu, M., Zachia, M., Belc, N., Manasia, T., Burnichi, F. and Israel-Roming, F., 2019. Effect of boiling on the antioxidant potential of cabbage varieties. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Food Science and Technology*, 76(2), pp.149-153.

Nursini, N.W., Venti, K.L. and Wita, I.G.A. (2020) 'Antioxidant Activity, Fiber, Acidity (pH), and Population of Lactic Acid Bacteria in Green Juice as Functional Beverages', *Digital Press Life Sciences*, 2, p. 00005. Available at: <https://doi.org/10.29037/digitalpress.22331>.

Pizzino, Gabriele., Irrera, Natasha., Cucinotta, Mariapaola., Pallio, Giovanni., Mannino, Federica., Arcoraci, Vincenzo., Squadrito, Francesco., Altavilla, Domenica., and Bitto, Alessandra. (2017) 'Oxidative Stress: Harms and Benefits for Human Health', *Oxidative Medicine and Cellular Longevity*. Hindawi Limited. Available at: <https://doi.org/10.1155/2017/8416763>.

Pruteanu, Lavinia Lorena., Bailey, David Stanley., Grădinaru, Andrei Cristian., and Jäntschi, Lorentz. (2023) 'The Biochemistry and Effectiveness of Antioxidants in Food, Fruits, and Marine Algae', *Antioxidants*. MDPI. Available at: <https://doi.org/10.3390/antiox12040860>.

Sanda, Andrei., Andreea, Bunea., D., A., Dumitraș., Adela, Pinte. (2019). Comparative Study of the Antioxidants Compounds in Fresh and Thermally Processed Tomatoes Juice. *Bulletin of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca*, 76(1):63-69. doi: 10.15835/BUASVMCN-FST:2019.0010.

Shivani, Sud. (2022). Antioxidant Activities and Phenolic Acids in Different Raw and Boiled Potatoes and Sweet Potatoes. doi: 10.31390/gradschool_theses.3849

Tuğba, İ.D.U.Ğ., GÜLDEMİR, H.H., Ali, Ş.E.N. and GÜLDEMİR, O., 2022. Investigation on the effects of cooking methods on anti-inflammatory and antioxidant activities of five mostly consumed vegetables in winter. *International Journal of Agriculture Environment and Food Sciences*, 6(1), pp.182-188.

Wang, Z., Shi, J., Sun, S., Zhu, L., He, Y., Jin, R., Luo, L., Zhao, L., Peng, J., and Zhou, Z. (2022) 'Estimation of Dry Matter and N Nutrient Status of Choy Sum by Analyzing Canopy Images and Plant Height Information', *Remote Sensing*, 14(16). Available at: <https://doi.org/10.3390/rs14163964>.

WHO. (2023). Non Communicable Disease. Diambil kembali dari World Health Organization: <https://www.who.int/home/search?indexCatalogue=genericsearchindex1&searchQuery=noncommunicable%20disease&wordsMode=AllWords>

Zou, L., Tan, W., Du, Y., Lee, H., Liang, X., Lei, J., Striegel, L., Weber, N., Rychlik, M., and Ong, C. (2021) 'Nutritional metabolites in Brassica rapa subsp. chinensis var. parachinensis (choy sum) at three different growth stages: Microgreen, seedling and adult plant', *Food Chemistry*, 357. Available at: <https://doi.org/10.1016/j.foodchem.2021.129535>.