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Effect of water from various sources on bioactive contents, antioxidant activities, and physical characteristics of cooked broccoli

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Abstract

The present work aimed to determine the influence of various water sources on the bioactive contents, antioxidant activities, and physical characteristics of cooked broccoli. The boiling process was conducted with eight water sources at 0, 4, and 6 min. The water used included bottled water (BW), non-reverse osmosis (RO) refilled water, RO refilled water, municipal waterworks of Bogor city, municipal waterworks of Jakarta city, groundwater of Jakarta city, dense population groundwater of Bogor city, and less dense population groundwater of Bogor city. The water samples were tested following the Indonesian standards for drinking water released by the Ministry of Health. Four water samples did not meet the standard: dense population groundwater of Bogor city for pH and nitrate value, groundwater of Jakarta city for total dissolved solid, and less dense population groundwater of Bogor city and RO water for pH only. The broccoli samples were boiled using the water samples, and showed different physical and chemical properties. The broccoli samples cooked using the water samples with high pH (> 7.00) exhibited softer texture. Two best DPPH IC_{50} antioxidant activities were obtained from the broccoli samples cooked for 4 min in the RO (93 mg solid part/mL) and BW (117 mg solid part/mL) samples. The broccoli samples cooked in BW for 4 min contained the highest vitamin C content, whereas the vitamin C contents of the broccoli samples cooked in the municipal waterworks and the groundwater of Jakarta city were fully degraded. The vitamin C contents in the cooked broccoli samples exhibited higher contribution as antioxidants compared with phenolics.

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Introduction

Water quality is an important aspect in food supply because water is the most extensively used raw material during food processing (Shrivastava *et al.*, 2022). Water from different sources may have different qualities as water quality is determined by many factors, including the environmental condition of the water source (Mainali and Chang, 2018; 2021; Chathuranika *et al.*, 2023). In Indonesia, there are several governmental standards for water quality, especially for drinking water. Based on those standards, the quality parameters of water can be grouped into physicochemical parameters (temperature, pH, dissolved oxygen, dissolved solids, and minerals), micropollutants (organic and inorganic compounds, and pesticides), and microbiological parameters. When using water for food processing, water quality is a crucial factor to be considered. One of the common food processes conducted in food services and household-scale cooking processes is boiling. During boiling, water quality affects the chemical characteristics of the boiled food (Sawangjang and Takizawa, 2023).

Vegetables are commonly used as ingredients that are processed by boiling in food and household-scale cooking processes. Broccoli is a vegetable that is often processed in cooking facilities because it has high nutrients and a delicious taste (Czarnowska-Kujawska *et al.*, 2022). Broccoli also contains an abundant concentration of bioactive compounds, such as ascorbic acid, phenolic, and flavonoids, which

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show antioxidant activity (Lv *et al.*, 2017; Xu *et al.*, 2020). Nutritional and bioactive compounds in broccoli can degrade during cooking because many of the compounds are sensitive to high temperatures (Xu *et al.*, 2020). The degradation of these compounds may be influenced by temperature, cooking duration, and the chemical compounds present in the water used for boiling. In terms of cooking duration, conventional boiling time for broccoli is approximately 4 min (Sanjuán *et al.*, 2001). Optimal time for cooking can minimise the loss of bioactive compounds, and maintain the antioxidant activity in food ingredients (Kao *et al.*, 2014).

In addition to the chemical characteristics, boiling can also change the physical characteristics of broccoli. Broccoli has an intense green colour owing to the presence of chlorophyll. During heating, chlorophyll is degraded into chlorophyll derivatives such as pheophytins, which impart a brown colour (Kusmita *et al.*, 2015; Gómez-Coca *et al.*, 2020). This degradation is accelerated in the presence of acid (low pH) (Indrasti *et al.*, 2018). Furthermore, the texture of broccoli softens after boiling, and this change is affected by pH value and the presence of mineral compounds in the water used for boiling (Mubaiwa *et al.*, 2019).

To the best of our knowledge, studies regarding the effect of water from different sources for boiling on the bioactive contents, antioxidant activities, and physical properties of broccoli have not been conducted before. Therefore, the present work was designed to analyse the characteristics of water from different sources. The results of the analyses could determine which water meets the Ministry of Health Republic Indonesia (Kemenkes, 2023) standards. Samples from all water sources were used to boil the broccoli to evaluate their effect on the quality of cooked broccoli, including the effect of water that did not meet the standards. The present work thus aimed to investigate the effect of water characteristics from various sources on the chemical (vitamin and bioactive content) and physical qualities of boiled broccoli. The present work would provide information regarding the possibility to retain the quality of cooked broccoli (especially its colour, nutrition, bioactive compound, and antioxidant activity) by choosing a water source for cooking. This information is important for food handlers in food services, and for domestic applications to serve cooked broccoli with better colour and retained nutrition and bioactive compounds.

Materials and methods

Water sources and broccoli samples

The water samples used in the present work included bottled water (BW), non-reverse osmosis (RO) refilled water, RO refilled water, municipal waterworks of Bogor city, municipal waterworks of Jakarta city, groundwater of Jakarta city, dense population groundwater of Bogor city, and less dense population groundwater of Bogor city. Water sampling was conducted in accordance with the water sampling method from SNI 8995 2021 (SNI, 2021). Water was sampled in 19-L sealed gallons containers. The water samples were stored in a room with air-conditioning (18 - 20°C). The water samples were also checked for pH, during storage and before use, to ensure the quality. Fresh broccoli samples were obtained from a broccoli plantation (Agri Packing House, Lembang, West Java, Indonesia). Broccoli samples were picked from the same garden at a harvest age of 60 d.

Chemicals

The chemicals used in the present work were methanol, 2,2-diphenyl-2-picryl-hydrazyl (DPPH), ascorbic acid standard, ethanol, Folin-Ciocalteu reagent, Na_2CO_3, gallic acid standard, high-performance liquid chromatography (HPLC)-grade water, and HPLC-grade methanol. All chemicals were of analytical grade, and obtained from Merck (Germany).

Sample preparation

Fresh broccoli samples were cut evenly with a stem height of 5 - 7 cm, and a flower width of 3 - 4 cm. The broccoli floret pieces were divided into three categories based on their size, large (13.70 - 19.30 g/piece), medium (8.78 - 10.20 g/piece), and small (5.03 - 6.04 g/piece). For each treatment, the large, medium, and small pieces of broccoli were used in the same proportion (four large cuts, five medium cuts, and eight small cuts) to reach a total of 150 g of broccoli sample. This was done to avoid bias caused by the size of the broccoli piece on bioactive content, antioxidant activity, and physical characteristics of cooked broccoli samples. The broccoli pieces were then wrapped in a sealed container, and stored in the refrigerator for a maximum of 24 h. The broccoli pieces (150 g) were subjected to boiling treatment using each type of water sample, with the ratio of broccoli:water being 1:10. The broccoli pieces were immersed in boiling water (95°C) until completely submerged. Boiling was conducted for 4 and 6 min. In addition, the broccoli pieces boiled for 2 min was used for colour and texture analyses only. After boiling, the broccoli pieces were soaked in cold water (± 18°C, 5 min), following which the water was drained, and the broccoli pieces were ready for texture and colour analyses shortly after boiling. Boiling was conducted in triplicate. The boiled broccoli for chemical analysis was dried in a freeze-dryer, and then ground into a fine powder using a blender. For each chemical analysis, 5 g of dried broccoli was dissolved in 50% methanol, and stirred using a magnetic stirrer for 5 min. The volume was adjusted to 100 mL using 50% methanol, and filtered using a filter paper. The filtrate was used for chemical analysis. Raw broccoli was also freeze dried and analysed as a control.

Analysis of general characteristics of water

The eight types of water samples were analysed using the parameters prescribed in the Regulation of the Ministry of Health of the Republic of Indonesia (Kemenkes, 2023), namely microbiological, physical, and chemical parameters.

Minerals analysis of water

The mineral content of the eight types of water samples were analysed, which included calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), chloride (Cl), phosphate (F), iron (Fe), and phosphorus (P), using inductive coupled plasma mass spectrometry (ICP/MS) following the Standard Methods for the Examination of Water and Wastewater (APHA, 2012). These analyses were conducted in a national accredited laboratory.

Colour analysis of broccoli

Colour analysis was conducted following a method described by Mashiane *et al.* (2021), with slight modifications, using a chromameter (CR-400, Konica Minolta, Osaka, Japan). The measured parameters were L*, a*, and b*. L* indicates brightness, a* indicates a mixed chromatic colour between red (+a) and green (-a), and b* indicates a mixed chromatic colour between blue (-b) and yellow (+b). The boiled broccoli pieces were attached to the sensor of the tool, and light was focused on the sample, and the amount of reflection was captured using a chromameter sensor. The values of L*, a*, and b* displayed on the device were then recorded. The analysis was conducted thrice at different points, and the obtained L*, a*, and b* values were averaged. Raw broccoli served as a control.

Texture analysis

Texture analysis was conducted based on a method described previously (He and Xiao, 2018) using a texture analyser (TAXt2i; Stable Micro system, UK). This test used a 6-mm cylindrical probe with a trigger load of 103 g and a test speed of 10 mm/s. Two compressions with a depth of 6 mm were performed at the same point. The instrument records speed in distance per second. The parameters calculated from the texture analyser were hardness (g) and brittleness (g). The part of the boiled broccoli used for the analysis was the middle of the stem. Raw broccoli served as a control.

Vitamin C analysis

Vitamin C analysis was conducted using HPLC following a method described previously (Iori *et al.*, 2004). The broccoli samples (methanolic extract) obtained from a sample preparation of 20 μL were analysed in an HPLC instrument with the following specifications: UV detector at a wavelength of 261 nm and Kromasil column of C-18 (25 × 0.4 cm; 5 m particle size). The mobile phase comprised 0.1% acetic acid and methanol (95:5, v/v), and the flow rate was 0.9 mL/min. The results were calculated using a calibration curve of ascorbic acid, and expressed as µg/g broccoli solid part.

DPPH antioxidant analysis

A 2,2-diphenyl-2-picryl-hydrazyl (DPPH) IC_{50} antioxidant analysis was conducted following a previously described method (Vignoli *et al.*, 2011). The DPPH solution was prepared by dissolving 2.5 mg of DPPH in 25 mL of 96% ethanol. The solution was homogenised and kept at room temperature (26 - 30°C) for 30 min before use. A series of dried broccoli extracts (5, 2.5, 2, 1.25, and 1 g eq./100 mL) were prepared. Then, 10 μL from each extract was placed into a vial, and 1 mL of 100 mM acetate buffer at pH 5.5, 1 mL of 96% ethanol, and 0.5 ml of 250 μM ethanolic DPPH solution were added to the vial. The sample was vortexed for 20 s, and then allowed to stand for 15 min before being tested. Antioxidant analysis was performed using an ultraviolet-visible (UV-Vis) spectrophotometer with an absorbance of 517 nm in a dark room (triplicate). The DPPH radical inhibition (%) was then calculated using Eq. 1:

% Inhibition =

$$\frac{control\ absorbance\ value - extract\ absorbance\ value}{control\ absorbance\ value} \times 100\% \quad \text{(Eq. 1)}$$

The IC_{50} value was determined by calculating the 50% inhibitory concentration from the linear regression equation of the DPPH radical inhibition (%) by broccoli extract at different dilutions.

Total phenolic content analysis

Total phenol content (TPC) analysis was conducted following a previous method (Jin *et al.*, 2015). In a test tube, 0.1 mL broccoli extract was placed, following which 0.4 mL of 95% methanol and 2.5 mL of 10% Folin-Ciocalteu reagent was added to the tube. The mixed solution was left for 5 min, and then 2.5 mL of 7.5% Na_2CO_3 was added to the mixture, and vortexed. The samples were incubated for 45 min at 45°C, and then the absorbance was measured at 765 nm. A series of standard solutions of gallic acid at a concentration range of 30 - 100 mg/L (triplicate) was used for quantification. The TPC of broccoli was expressed as µg GAE/g solid part.

Statistical analysis

Data processing, including antioxidant DPPH IC_{50}, TPC, vitamin C, and minerals, was conducted using analysis of variance and Duncan's advanced test at 5% level using SPSS software and Microsoft Excel. Data on the physical properties, including texture and colour, were analysed using principal component analysis (PCA) using XLSTAT software (Addinsoft, Paris, France).

Results and discussion

Chemical characteristics of water samples

The chemical characteristics of the eight water samples used in the present work are presented in Table 1. Water sources that met the requirements of the Indonesian regulation (Kemenkes, 2023) included BW, non-RO water, municipal waterworks of Bogor city, and municipal waterworks of Jakarta city, whereas the other four samples did not meet the requirements. The dense population groundwater of the Bogor city sample exhibited the highest nitrate content, which was 60.3 mg/L, exceeding the maximum limit (max. 50 mg/L), and the lowest pH value was 4.57 below the minimum limit (6.5 - 8.5). The less dense population groundwater of Bogor city and RO water samples also did not meet the requirements because they had pH values below the minimum limit of 6.19 and 5.87, respectively. The groundwater of Jakarta city sample had the highest total dissolved solids (TDS) value of 629 mg/L, which exceeded the standard requirements (max. 500 mg/L). In addition to chemical characteristics, the physical and microbiological characteristics of water samples were investigated.

The nitrate contamination in the dense population groundwater of Bogor city sample was because this sample was obtained from a densely populated housing area. Changes in soil function in the location of settlements cause nitrate contamination in groundwater. This is due to high solubility of nitrate in the soil which causes nitrate to elevate to the surface (Keeler and Polasky, 2014). Another possibility is the low sanitation of the densely populated settlements, which make the construction of septic tanks difficult. A previous study reported that the location of water wells containing nitrate at > 50 mg/L is < 10 m from the septic tank (Fathmawati *et al.*, 2018).

The groundwater of Jakarta city water sample contained the highest mineral content among all the water samples, followed by the greatest TDS value. TDS is the combined value of all inorganic and organic materials or salts found in water, such as calcium, magnesium, and potassium, as well as anions, such as carbonate bicarbonate, nitrate, chloride, and sulphate (Li *et al.*, 2021). Water with a low TDS value usually has undergone distillation or deionisation (Islam *et al.*, 2016). The groundwater of Jakarta city was obtained from well water whose chemical content was influenced by geochemical conditions in the soil and human activities. As this well water was located in a densely populated housing, the groundwater of Jakarta city water may be contaminated by human activities. In addition, human activities can increase contaminant levels in groundwater (Li *et al.*, 2021). The RO water sample had the least mineral content followed by TDS value. RO is a water filtration process that uses a high-pressure membrane to filter all chemical components in water until it is free from minerals (Vingerhoeds *et al.*, 2016).

The pH value measures the relative amounts of free hydrogen and hydroxyl ions. Water that has more free hydrogen ions is acidic, whereas water

Table 1. Chemical quality characteristics of eight water from different sources, and their conformity with Ministry of Health Republic Indonesia (Kemenkes) Regulation No. 2/2023.

Parameter	Unit	Permitted level*	Complied with Kemenkes Regulation No. 2/2023				Did not comply with Kemenkes Regulation No. 2/2023			
			Bottled water	Non-reverse osmosis water	Municipal waterworks of Bogor city	Municipal waterworks of Jakarta city	Groundwater of Jakarta city	Dense population groundwater of Bogor city	Less dense population groundwater of Bogor city	Reverse osmosis water
Calcium	mg/L	-	13.60 ± 0.17^{e}	11.57 ± 0.25^{d}	7.13 ± 0.04^{b}	11.23 ± 0.49^{d}	37.67 ± 1.19^{f}	8.84 ± 0.37^{c}	14.10 ± 0.81^{e}	0.39 ± 0.02^{a}
Magnesium	mg/L	-	5.16 ± 0.12^{d}	4.22 ± 0.15^{c}	2.05 ± 0.03^{b}	2.46 ± 0.13^{b}	23.30 ± 0.81^{e}	4.21 ± 0.19^{c}	5.06 ± 0.30^{d}	0.18 ± 0.03^{a}
Sodium	mg/L	Max 200	6.77 ± 0.27^{b}	5.20 ± 0.39^{b}	7.55 ± 0.21^{bc}	5.14 ± 0.34^{b}	86.90 ± 2.86^{e}	9.29 ± 2.16^{c}	6.70 ± 0.33^{b}	1.38 ± 0.52^{a}
Potassium	mg/L	-	1.99 ± 0.07^{cd}	2.32 ± 0.51^{d}	1.76 ± 0.04^{c}	2.24 ± 0.16^{d}	14.77 ± 0.20^{e}	0.57 ± 0.22^{b}	1.94 ± 0.09^{cd}	0.15 ± 0.04^{a}
Chloride	mg/L	Max 250	n.d.	10.93 ± 0.5^{1}	n.d.	12.43 ± 0.55	49.07 ± 0.37	21.77 ± 0.55	9.31	n.d.
Iron	mg/L	Max 0.1	n.d.	n.d.	0.06 ± 0	n.d.	n.d.	n.d.	n.d.	n.d.
Sulphide	mg/L	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Phosphate	mg/L	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Nitrate	mg/L	Max 50	5.34**	5.04**	3.97**	10.6**	19.2**	60.3**	25.5**	4.12**
TDS	mg/L	Max 500	108**	94**	66**	90**	629**	131**	119**	11**
Hardness	mg/L	Max 500	56**	46**	28**	36**	184**	42**	53**	1.8**
Sulphate	mg/L	Max 250	1.1**	n.d.**	18**	12**	46**	0.7**	1.7**	n.d.**
pH		6.5 - 8.5	7.12 ± 0.03^{f}	7.02 ± 0.06^{e}	6.66 ± 0.05^{d}	7.00 ± 0.03^{e}	7.50 ± 0.01^{g}	4.57 ± 0.05^{a}	6.19 ± 0.03^{c}	5.87 ± 0.01^{b}

TDS = total dissolved solid. Values are mean ± SE of three replications (n = 3). Means followed by different lowercase superscripts in similar row are significantly different among samples ($p < 0.05$). (*) Ministry of Health Republic Indonesia Regulation No. 2/2023. (**) Data are mean of two replicates (n = 2). n.d. = not detected (the value was below the Limit of Detection).

containing more free hydroxyl ions is basic. The groundwater of Jakarta city exhibited the highest mineral content followed by a high pH value. Likewise, the RO water sample demonstrated the lowest mineral content and a low pH value. Based on a previous study (Vingerhoeds *et al.*, 2016), water with low pH tends to have low mineral components followed by low TDS values. As pH can be affected by the chemical components present in water, it is important to measure pH as an indicator of chemical changes in the water. Water needs to be assessed in terms of constant levels and relative proportions of minerals and geological elements at the point of origin to account for cycles of natural fluctuations (Faysal *et al.*, 2017). The pH value is directly proportional to the mineral content of water, and may influence the physical and chemical properties of boiled broccoli.

Effects of different sources of water for cooking on texture and colour of cooked broccoli

The textures and colours of broccoli boiled with eight different types of water and at different boiling durations (2, 4, and 6 min) are presented in Figure 1. The texture and colour of the broccoli were affected by the heating duration and the characteristics of the water sources. Longer heating durations decreased hardness (Figure 1A) and brittleness (Figure 1B). The effect of water sources and heating duration on the colour of cooked broccoli did not exhibit any pattern alongside the effect of water source on broccoli texture (Figure 1C). To obtain a more comprehensive understanding, the data were analysed using biplot PCA, as presented in Figure 2.

The control sample (fresh broccoli; sitting in quadrant B) was the hardest compared with cooked broccoli. Broccoli cooked with BW, groundwater of Jakarta city, and municipal waterworks of Jakarta city for 6 min (sitting in quadrants C and D) exhibited softer texture and lower brittleness values. BW, groundwater of Jakarta city, and non-RO water sources exhibited higher pH and bivalent minerals, as presented in Table 1. These data showed that water with pH of ≥ 7 and high bivalent mineral content imparted cooked vegetables with a softer and less brittle texture.

The presence of water with a high pH and mineral content can accelerate the cooking time. Monovalent (Na^+ and K^+) and divalent (Mg^{2+} and Ca^{2+}) cation minerals can help enhance water absorption capacity, and reduce food hardness (Mubaiwa *et al.*, 2019). Texture changes are strongly influenced by the nature of the material, processing conditions, ions, and enzymes that are present in different parts of the cells, and interact with each other during heating. In vegetables and fruits, thermal processing causes a marked degradation in polysaccharide pectin, resulting in reduced intercellular adhesion, and consequently increasing softening (Andrés-Bello *et al.*, 2013). The presence of high amounts of calcium in the groundwater of Jakarta city water was related to the pectin content in broccoli, which was responsible for soft texture. Calcium has been hypothesised to occur in association with anionic groups such as oxalate (Salgado *et al.*, 2023).

PCA biplot also discriminates the colour of the broccoli samples into four quadrants. The control sample (fresh broccoli) exhibited a different colour from all the cooked samples because it was separated from the cooked sample. Almost all the broccoli samples cooked with RO and dense population groundwater of Bogor city water were in quadrant A. These samples exhibited a more intense b* value (yellow colour) compared with the other samples. The RO and dense population groundwater of Bogor city water samples exhibited a low pH (< 6.00), as presented in Table 1. Chlorophyll degradation may cause colour change in cooked broccoli, and this degradation is elevated with high temperature and acidity (low pH) (Indrasti *et al.*, 2018; Gómez-Coca *et al.*, 2020). Therefore, the samples were divided into two groups by axes F1. Group A comprised all water sources except Jakarta city, and group B comprised water sources from Jakarta city. Group A was dominated by drinkable water, signifying that the water had undergone treatment. Meanwhile, the result of municipal waterworks and groundwater of Jakarta city showed the existence of a phenomenon on water sources in Jakarta. Based on the data shown in Table 1, the pH and chloride content of the municipal waterworks and groundwater of Jakarta city remained high. The result may affect the physical and chemical properties of cooked broccoli.

Effects of different sources of water for cooking on TPC, vitamin C, and antioxidant activity of cooked broccoli

The effect of different sources of water for

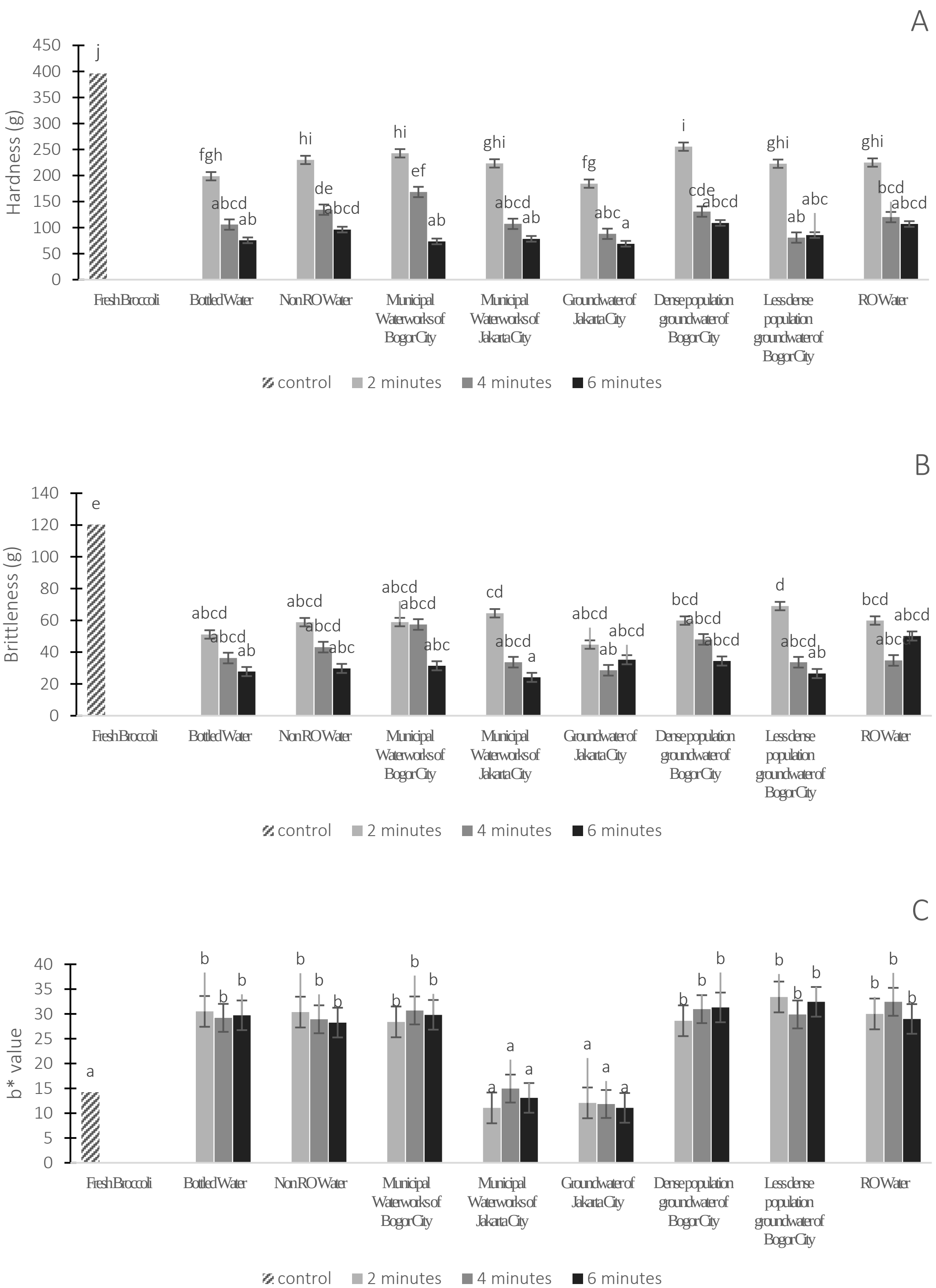

Figure 1. Effect of water sources for cooking on hardness **(A)**, brittleness **(B)**, and b* value **(C)** of cooked broccoli samples. Values are mean ± SE of three replications (n = 3). Means with different lowercase letters are significantly different among samples ($p < 0.05$).

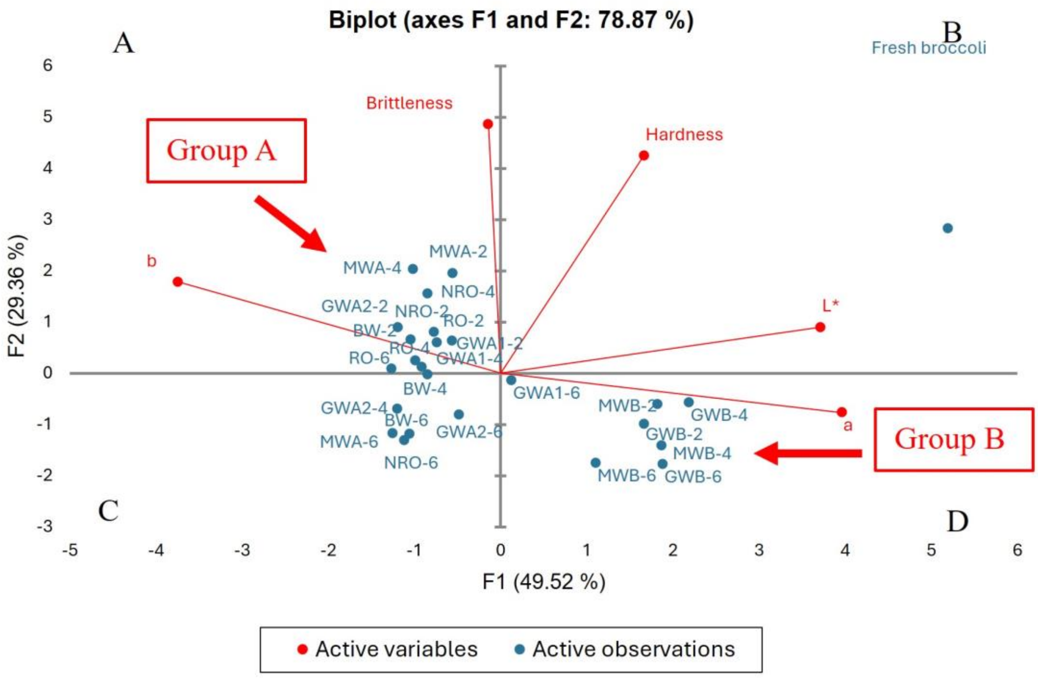

Figure 2. PCA biplot of physical properties (texture and colour) of cooked broccoli samples with eight water sources. BW: bottled water; NRO: non-reverse osmosis refilled water; RO: reverse osmosis refilled water; MWA: municipal waterworks of Bogor city; MWB: municipal waterworks of Jakarta city; GWB: groundwater of Jakarta City; GWA1: dense population groundwater of Bogor City; and GWA2: less dense population groundwater of Bogor city. The last digit of sample code represents boiling duration: 2, 4, and 6 min.

cooking on the TPC and vitamin C of cooked broccoli is presented in Figure 2. The sources of the water samples had a significant effect ($p < 0.05$) on the TPC (Figure 2A). The highest TPC was in the non-RO of 4 min water sample at 7,327 µg GAE/g solid part. The TPC in the boiled broccoli sample were higher compared with those in the fresh broccoli control sample. Similar results were also reported by a previous study, where steaming and microwave treatment increased the TPC of broccoli (Kim *et al.*, 2021). The increase in TPC in cooked samples was possible because of intense cell wall damage, and release of phenolic compounds trapped in the fibre (Adefegha and Oboh, 2011). Boiling broccoli for 6 min decreased the TPC compared with the 4 min of boiling. Zhang and Hamauzu (2004) reported the loss of TPC of up to 71.9 and 71.6% in broccoli after 5 min of boiling and microwave heating, respectively.

The groundwater of Jakarta city sample exhibited the lowest TPC compared with another cooked broccoli. This sample was boiled with water containing the highest mineral content, pH, and TDS. Chemical components in the groundwater of Jakarta city water might have had an influence on the degradation of phenol levels in broccoli. The increased surface area of the tissue in contact with cooking water, cooking temperature, and a long cooking duration tend to disrupt cell walls and damage phenolic compounds (Porter, 2012). In addition, the groundwater of Jakarta city contained the highest amount of TDS, representing the level of contamination. Some of these dissolved solids may also accelerate the degradation of phenolic compounds. Phenolics are antioxidant compounds (Ludwig *et al.*, 2012) sensitive to oxidation triggered by any radical or pro-oxidant that may be present in contaminated water. Meanwhile, broccoli cooked with RO water also exhibited a relatively high TPC (7,076.23 g/g solid part). The characteristics of RO water with a low pH indicated that acidic conditions could retain phenolic compounds (Altunkaya and Gökmen, 2008).

The different sources of water also had a significant effect ($p < 0.05$) on the vitamin C content of cooked broccoli (Figure 2B). The control sample (fresh broccoli) exhibited a lower content than the cooked samples. The increase in vitamin C content in cooked vegetables could be attributed to the increased extractability of ascorbic acid from vegetable extracts owing to the softening of the matrix due to heating.

Lee *et al*. (2018) reported that steaming and microwave treatment increased the vitamin C content of broccoli.

The highest vitamin C content was exhibited by the broccoli samples cooked in BW for 4 min (876.5 mg/kg solid part). BW used for cooking this broccoli sample met the Ministry of Health Republic Indonesia standards for drinking water. It also had a moderate pH and mineral content compared with the other water sources. Each water sample from a different source contained a different number of chemical components. This was influenced by the geological conditions and treatment of the water sources; thus, the amount of minerals in the water were different. Neutral and alkaline pH conditions affect the stability of vitamin C, where the higher the pH, the more unstable the vitamin C (Pathy, 2018). With the same boiling time of 4 min, the vitamin C content in the groundwater of Jakarta city (pH 7.5) was completely degraded despite this water source not meeting the Ministry of Health Republic Indonesia standards for TDS.

The degradation of vitamin C in the sample was accelerated by heating and the presence of oxidising compounds. As earlier explained, groundwater of Jakarta city water was obtained from a densely populated housing area that may be contaminated by various chemical contaminants owing to human activities, as shown by its TDS value of 629 mg/L, which did not meet the Ministry of Health Republic Indonesia standards. Those contaminants may accelerate the degradation of vitamin C during heating. However, municipal waterworks of the Jakarta city sample also imparted cooked broccoli with the lowest vitamin C levels, warranting more investigation. Data to discuss this are not yet unavailable from the literature.

Vitamin C levels after 6 min of cooking decreased compared with those after 4 min of cooking. Cooking for 4 min may increase the extractability of vitamin C, but further heating led to vitamin C degradation. Vitamin C is highly sensitive to oxidation, and the oxidation of vitamin C and other reactions during heating is highly dependent on various factors. Cooking (high temperatures treatment) and other preparations caused significant vitamin C loss in broccoli owing to oxidation reaction (Soares *et al.*, 2017; Giannakourou and Taoukis, 2021).

Different sources of water exhibited a significant effect ($p < 0.05$) on the DPPH IC_{50} antioxidant activity of boiled broccoli (Figure 3C). Antioxidant activity in the control sample (fresh broccoli) demonstrated a lower activity than the boiled broccoli sample. This agreed with the results of a previous study where boiled broccoli exhibited a higher antioxidant activity than the fresh one (Wachtel-Galor *et al.*, 2008). The increase in antioxidant activity after cooking may be attributable to the increase in the extractability of antioxidant compounds. This was supported by the data of antioxidant compounds, where cooked samples contained more TPC and vitamin C than fresh broccoli.

The highest antioxidant activity was found in the broccoli cooked in RO and BW for 4 min, at 92.9 and 115.79 mg eq./mL, respectively. The data from Figure 2 support this finding, where samples obtained from broccoli boiled for 4 min in BW and RO water samples exhibited higher amounts of TPC and vitamin C than the other samples. Vitamin C also acts as an antioxidant in broccoli. Vitamin C is a strong antioxidant compound, with a DPPH IC_{50} of 6.1 µg/mL (Nariya *et al.*, 2013). Herein, ascorbic acid was used as a positive control for the DPPH IC_{50} test of 270 µg/mL. In addition, our previous study reported that phenolics, especially caffeoylquinic acid, inhibited DPPH radicals (Herawati *et al.*, 2019).

The broccoli sample boiled in the groundwater of Jakarta city water and dense population groundwater of Bogor city water exhibited lower antioxidant activity. The groundwater of Jakarta city water did not meet the TDS standard, whereas the dense population groundwater of Bogor city water did not fulfil the nitrate standards. Contaminants in the water samples may affect the antioxidant activity of boiled broccoli. In addition, the standard error of the samples was high, indicating that the data were inconsistent. Inconsistency in antioxidant activity data is usually found in less active samples.

PCA biplot of chemical properties correlation of cooked broccoli

The PCA biplot of the chemical properties of cooked broccoli is presented in Figure 4 to emphasise the association of these parameters (TPC, vitamin C, and DPPH IC_{50}). The samples cooked in the groundwater of Jakarta city for 4 and 6 min were on the right of the F1 axis (quadrants B and D). Both these samples exhibited a high pH and TDS that led to high mineral content (Table 1). This along with the DPPH variable, which was also located to the right of

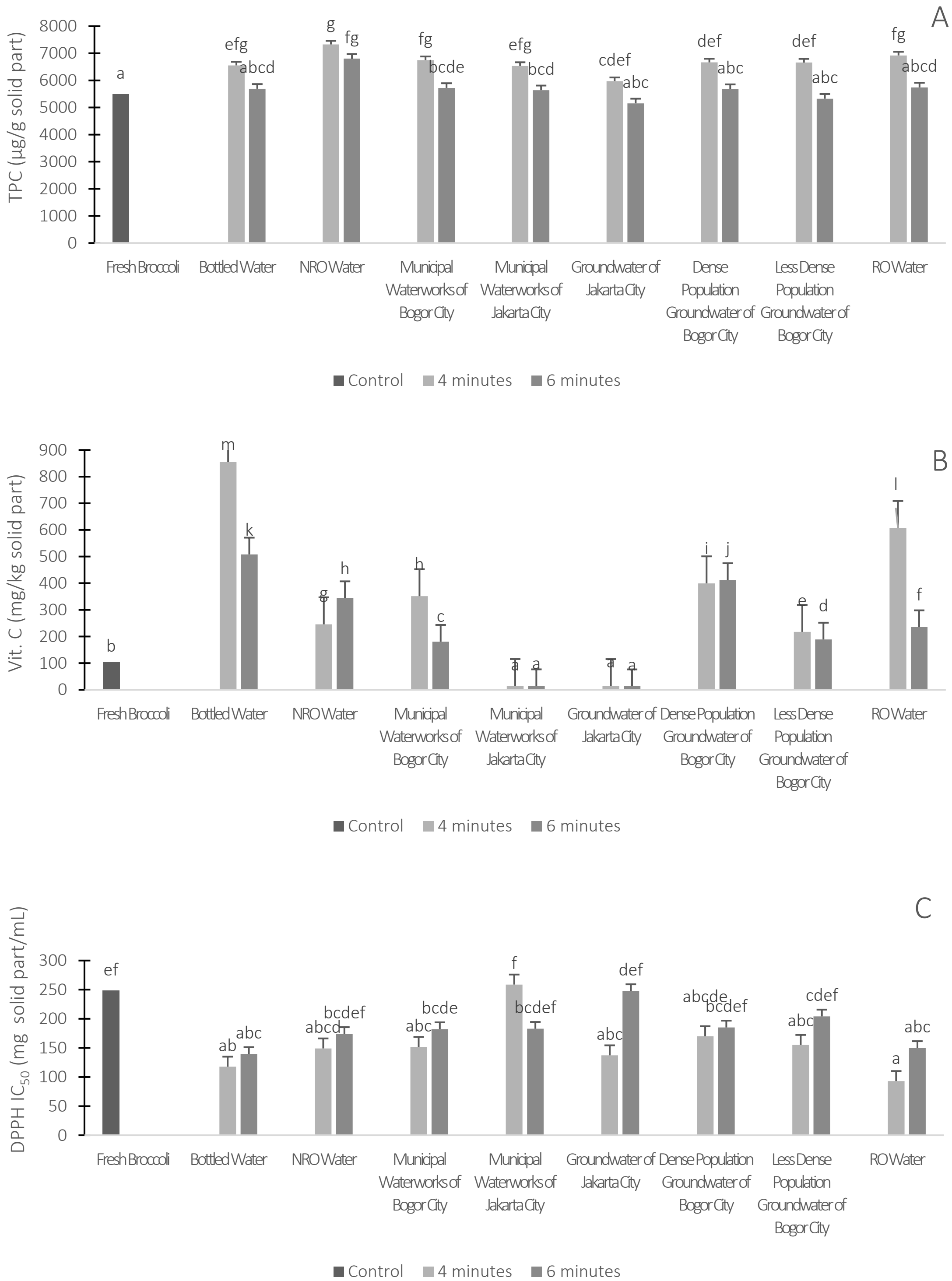

Figure 3. Effect of water sources for cooking on total phenolic content (TPC) **(A)**, vitamin C **(B)**, and DPPH IC_{50} **(C)** of cooked broccoli samples. Values are mean ± SE of three replications ($n = 3$). Means with different lowercase letters are significantly different among samples ($p < 0.05$).

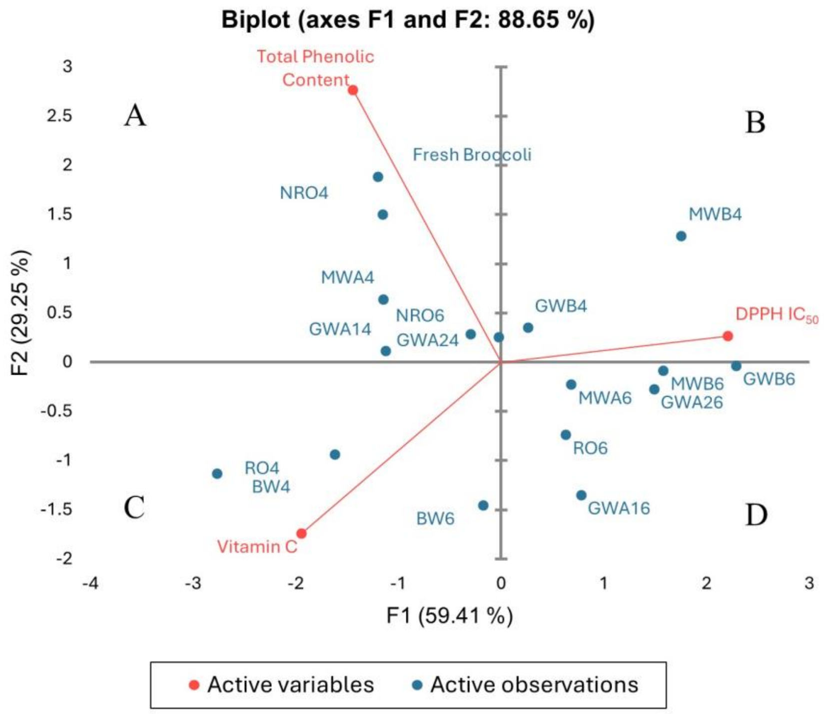

Figure 4. PCA biplot of chemical properties of water (minerals and pH) and cooked broccoli samples. BW: bottled water; NRO: non-reverse osmosis refilled water; RO: reverse osmosis refilled water; MWA: municipal waterworks of Bogor city; MWB: municipal waterworks of Jakarta city; GWB: groundwater of Jakarta City; GWA1: dense population groundwater of Bogor City; and GWA2: less dense population groundwater of Bogor city. The last digit of sample code represents boiling duration: 2, 4, and 6 min.

the F1 axis, shows that the broccoli sample cooked in the groundwater of Jakarta city water exhibited the highest DPPH IC_{50} value; thus, this sample demonstrated the lowest antioxidant activity. However, the chemicals in the groundwater of Jakarta city water included minerals and chemicals owing to contamination due to human activities, as earlier explained. The result in Figure 4 is consistent with that in Figure 2, where the municipal waterworks of Jakarta city and groundwater of Jakarta city may have specific characteristics. The low antioxidant activity specifically in these samples may have occurred because of the high level of contaminants present in the water, which caused the degradation of the broccoli antioxidant compounds. These results proved that water with a high mineral content does not necessarily indicate good quality. As seen in Figure 4, boiling in water with a high nitrate content (dense population groundwater of Bogor city) also imparted broccoli with a low vitamin C, TPC, and antioxidant activity (quadrant A). High nitrate content is the only parameter in water that indicates contamination. Other contaminants may reduce vitamin C, TPC, and antioxidant activity in broccoli boiled in this water.

Broccoli samples boiled in RO and BW, especially for 4 min (quadrant C), gave consistent results of the highest vitamin C and antioxidant activities. The broccoli cooked in RO and BW were positioned opposite each other in term of DPPH IC_{50} values, indicating that these two samples had the highest antioxidant activity. This aligned with the DPPH IC_{50} principle, where the smaller the IC_{50} value, the higher the antioxidant activity. Since RO water did not meet the Ministry of Health Republic Indonesia standards, BW can be used to cook broccoli as it preserves vitamin C, TPC, and antioxidant activity. Supported by the Pearson correlation test results with 0.011 significance ($p < 0.05$), vitamin C significantly contributed to the antioxidant content in broccoli compared with TPC. Vitamin C content correlated with the antioxidant activity of Brassicaceae leaves (Martínez-Sánchez *et al.*, 2008). The present work demonstrated that vitamin C was more responsible for the antioxidant activity in broccoli than TPC.

Conclusion

Four water samples did not meet the standard of drinking water: groundwater of Jakarta city (high TDS levels), dense population groundwater of Bogor city (high nitrate levels), less dense population groundwater of Bogor city (low pH), and RO water (low pH). Cooking broccoli samples in water with a high pH and containing bivalent minerals accelerated the softening of broccoli tissue. For colour parameters, water with low pH increased the yellow colour of the cooked broccoli samples. The municipal waterworks and groundwater of Jakarta city exhibited distinct characteristics compared with the other water sources. The highest TPC was found in the broccoli samples cooked in non-RO water. In addition, the highest vitamin C content was observed in the broccoli samples cooked in BW. The broccoli samples boiled in RO and BW exhibited the highest DPPH IC_{50} antioxidant activity. Vitamin C contributed the most to the antioxidant activity of the broccoli samples rather than the TPC.

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