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Bioaccessibility of Antioxidant Capacity of Wedang Uwuh a Traditional Indonesian Beverage by Gastrointestinal Digestion

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Abstract

Wedang uwuh is a traditional Indonesian beverage that contains natural antioxidants. Hence, it is generally consumed by people for its beneficial effects on health. The antioxidant compounds can exert their activity only after passing through the digestive system. This study aims to determine the effect of boiling time on the bioaccessibility of antioxidants in wedang uwuh beverages after the gastrointestinal digestion process. The bioaccessibility was evaluated using the bioaccessibility index (BI) of total phenolic content (folin–ciocalteu method) and antioxidant capacity (FRAP, DPPH and ABTS assay). The research found that lengthening of boiling time could increase significantly ($p<0.05$) the total phenolic content, as well as antioxidant capacity. The 15 minutes of boiling had the highest value for total phenolic content and antioxidant capacity before digestion. Otherwise, after passing through gastric and intestinal digestion, boiling for 5 minutes resulted in the highest total phenolic content and antioxidant capacity of wedang uwuh beverage. This was due to heat processing influencing the stability of phenolic and antioxidant compounds before and after digestion. The bioaccessibility of antioxidant and phenolic compounds in wedang uwuh beverage was higher in the 5 minutes boiling process than in the 15 minutes. The boiling process for 5 minutes resulted in the bioaccessibility index of wedang uwuh beverage were 32.25% for total phenolic content, 37.25% for FRAP, 25.88% for scavenging of radical DPPH• and 83.45% for scavenging of radical ionic ABTS•+. Hence, it was recommended to use a boiling time of 5 minutes to prepare a wedang uwuh beverage. This study found that pH conditions and enzymatic activity in gastrointestinal digestion decreased phenolic and antioxidant contents.

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Introduction

Currently, herbal drinks are in great demand due to increased public awareness of a healthy lifestyle. Herbal beverages contain phytochemical compounds that are good for health. Phytochemical compounds such as phenolic acids, flavonoids, carotenoids and phenolic compounds are a good source of natural antioxidants.[1] The content of antioxidant compounds in food can inhibit the oxidation process in the food itself or prevent diseases caused by oxidative stress.[2] Scientific research shows that daily consumption of herbal teas has potential health benefits, including lowering serum markers of diabetes, weight loss and increasing anti-oxidant activity.[3,4]

Wedang uwuh is an Indonesian traditional beverage that has been consumed for generations. Wedang uwuh comes from a mixture of several herbal, that are sappan wood, ginger, nutmeg leaves, cinnamon leaves, clove stems, and rock sugar. Consumption of wedang uwuh every day is believed to have health effects on the body.[5] Each ingredient in wedang uwuh contains antioxidants and other compounds that are beneficial to health. The ginger extracts (*Zingiber officinale*) contain gingerols, shogaols, and zingerone, which have high antioxidant activity.[6] Brazilin is a major homoisoflavonoid constituent of sappan wood (*Caesalpinia sappan* L) and has the potential as an antioxidant, anti-inflammation, and antibacterial.[7,8] Eugenol is a phenolic compound found in clove, nutmeg leave, and cinnamon, which has the ability to reduce ferric ions and radical scavenging activity.[9] Several studies have shown that wedang uwuh has great potential health benefits that are as an immunomodulator,[10] antioxidant,[11] and blood glucose levels control.[12]

The wedang uwuh beverage is usually prepared in traditional way i.e. boiling or brewing. The heating process and boiling time affect the amount of phenolic compounds and antioxidants extracted into the drink.[13] The previous study showed that different ways of preparing wedang uwuh resulted in different antioxidant capacities and total phenolics.[11] The highest total phenolic content and antioxidant activity of basil was achieved by boiling it for 5 min while the lowest total phenolic content and antioxidant activity of rosemary was obtained when the boiling time was 15 min.14 In beverage products, the temperature and length of time of heating will affect the phenolic and antioxidant components that can be extracted.[15] In addition, the heating process can cause chemical structural transformations that affect bioaccessibility.[16]

The antioxidant capacity calculated in food materials does not always describe the amount of antioxidant compounds available for absorption by the small intestine (bioaccessibility).[17] The environment along the digestive tract such as pH conditions and digestive enzyme activity can cause changes in the chemical structure of antioxidant compounds that affect the level of their ability as antioxidants and bioaccessibility.[18] Determination of the bioaccessibility can be evaluate by *in vitro* gastrointestinal digestion assay.[19] The researches using the gastrointestinal digestion model on vegetable juices,[20] fruit extracts,[21] rock tea,[22] and green tea infusions[15] showed that total phenolic content and antioxidant capacity changed after *in vitro* gastrointestinal digestion. However, there is no research on the effect of the boiling time process on the bioaccessibility of bioactive components in wedang uwuh beverage after gastrointestinal digestion. Therefore, this study evaluates the effect of boiling time on the bioaccessibility of antioxidants in wedang uwuh beverage after *in vitro* gastrointestinal digestion.

Materials and Methods

Materials

The wedang uwuh used in this study is a commercial product with the highest level of sales and has a good level of satisfaction from consumers. The analytical materials that are porcine pepsin from gastric mucosa 700 FIP-U g^{-1}) purchased from Merck, porcine pancreatic (800 U mL^{-1} according to trypsin activity), porcine bile extract, and other analytical chemicals were purchased from Sigma.

Praparation of Wedang Uwuh Beverage

This research used the wedang uwuh with composition as described in Table 1. One serving of wedang uwuh is boiled in 250 ml of boiling water for three different boiling times (5, 10 and 15 minutes). The boiling time was selected based on product preparation instructions and household practices. After boiling, the filtrates were separated and stored in the amber bottles at a temperature -20°C.

Table 1: Composition of wedang uwuh per serving size (5 grams)

Material (Dried herbs)	Amounts (g)	Percentage (%)
Gingers (Variety: emprit)	2.80	56.12
Sappan wood	0.98	19.60
Clove stems	0.36	7.29
Nutmeg leaves	0.31	6.12
Cinnamon leaves	0.30	5.97
Clove leaves	0.25	4.90

Total Phenolic Content Measurement

Folin-ciocalteu method was used to determine total phenolic content in the sample.[20] First, 0.2 mL of wedang uwuh beverage sample was reacted with 1.5 mL of folin–ciocalteu reagent (1:10 v/v with water) and incubated for 5 minutes. Afterwards, 1.31 mL of sodium carbonate solution (60 gL-1) was added to the solution and incubated at room temperature for 90 minutes. After the incubation is complete, the absorbance of the solution is measured at the wavelength of 725 nm. Blanks are made from solvents. The calibration standard curve (R2=0.996) used a standard gallic acid solution with a concentration of 0 – 500 ppm, and the data were presented in GAE/serving (mg of gallic acid equivalent per serving).

Ferric-ion (Fe^{3+}) Reducing Antioxidant Power Assay (FRAP)

Measuring the power of the sample to reduce metal ions was carried out using the FRAP method.[20,23] FRAP reagent was prepared by reacting 300 mM acetate buffer (pH3.6), 20 mM ferric chloride, and 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) prepared in 40 mM HCl, following proportion: 10:1:1, respectively. Then to calculate the antioxidant capacity, 25 µL sample of wedang uwuh beverage was reacted with 1mL reagent and 1 mL of warm water (37oC). The mixture was incubated for 4 minutes at 37°C. After incubation, the absorbance of the solution was measured at a wavelength of 593 nm. The blank was made from distilled water and reagent. Antioxidant capacity was presented as mg of Trolox equivalent (TE) per serving using the Trolox standard curve (R^2=0.976) with a concentration of 0 – 250 ppm.

DPPH• Radical Scavenging Activity Assay (DPPH)

Antioxidant properties were also determined by measurement the scavenging capacity against DPPH• free radical.[20,24] 0.1 mL of the wedang uwuh beverage sample was reacted with 3.9 mL of DPPH reagent (75 µM, in methanol). The mixture was incubated for 30 minutes at 37°C. After that, the absorbance was measured at wavelength 517 nm, and results were reported in mg of Trolox equivalent (TE) per serving. The blank was made from methanol and reagent. A calibration curve (R2=0.984) was used a solution of Trolox with the concentration of 0 – 250 ppm.

ABTS•+ Radical Cation Scavenging Activity Antioxidant Assay (ABTS)

The antioxidant capacity of samples was assessed by ABTS assay.[20,25] Briefly, to prepare the ABTS reagent, 44 µL of potassium persulfate (2.45 nM) as an oxidant was added to 2.5 mL of the ABTS solution (7 mM in 20 mM sodium acetate buffer, pH 4.5). The mixture was incubated for 12-16 hours to form a dark blue-green radical solution. Then the radical solution is diluted to have an absorbance value of 0.7±0.01 at wavelength 734 nm to form ABTS reagent. The antioxidant capacity was determined by mixing the wedang uwuh beverage sample (20 µL) and reagent (3 mL), followed by incubation in a water bath for 30 minutes at 30°C. Absorbance measurement was carried out at a wavelength of 734 nm. The results were converted to mg Trolox equivalent per serving using the Trolox standard curve (R^2=0.97) with concentration 0 – 250 ppm.

Antioxidant bioaccessibility assay

The measurement of the bioaccessibility of antioxidants in wedang uwuh beverage was carried out using *in vitro* gastric and intestinal digestion followed a protocol of harmonized INFOGEST.[26] Gastric digestion was simulated using pepsin enzyme and simulated gastric fluid (SGF), while intestine digestion was simulated using pancreatin solution, bile extract and simulated intestinal fluid (SIF). SGF and SIF were made from stock solutions at the same molarity indicated in the protocol[26] and shown in Table 2.

Table 2: The Composition of SGF and SIF stock solutions

Salt Solution	mol L^{-1}	SGF (pH 3)		SIF (pH 7)	
		Stock formulation to prepare 400 mL (mL)	Final concentration in sample (mmol L^{-1})	Stock formulation to prepare 400 mL (mL)	Final concentration in sample (mmol L^{-1})
$MgCl_2(H_2O)_6$	0.15	0.4	0.12	1.1	0.33
KH_2PO_4	0.5	0.9	0.9	0.8	0.8
KCl	0.5	6.9	6.9	6.8	6.8
$NaHCO_3$	1	12.5	25	42.5	85
NaCl	2	11.8	47.2	9.6	38.4
$NH_4(CO_3)_2$	0.5	0.5	0.5	-	-
$CaCl_2(H_2O)_2$	0.3	-	0.15	-	0.6

In the first stage, gastric digestion simulation was carried out by mixing 20 mL sample with 3.2 mL pepsin solution (25000 U mL^{-1}), 15 mL of SGF solution, 10 µL of $CaCl_2$ (0.3 M), and 1.39 mL of water then the pH was adjusted to pH 3.0 by adding HCl (1 M). The mixture was incubated at 37°C using a water bath shaker (200 rpm) for 2 h. After incubation was complete, 20 mL of gastric chyme was taken to enter the intestinal digestion stage, and the remaining solution was kept in a freezer (−20°C).

Before simulating intestinal digestion, the pH of the gastric chyme was adjusted to pH 7.0 by adding NaOH (1 M). Afterwards, 20 mL of gastric chyme solution was reacted with 11 mL SIF, 5 mL pancreatin solution (800 U mL-1 based on trypsin activity), 2.5 mL fresh bile extract (160 mM), 40 µL CaCl2 (0.3 M) and 1.31 mL of water. The solution was incubated at 37oC for 2 h in a water bath shaker (200 rpm). Intestinal digestion simulation was completed after incubation. Furthermore, to determine the dissolved fraction (bioaccessible fraction), 10 mL solution from intestinal digestion was taken to be centrifuged at 4900 rpm at 37°C and the supernatant was taken. The fractions generated at each digestion stage (gastric, intestine, and supernatant phase) were stored at −20°C for further analysis (total phenolic content, FRAP, DPPH and ABTS). The bioaccessibility index (BI) value was measured with the following equation.[27]

BI (%) = B/A x 100, where A is total phenolic content or antioxidant capacity of wedang uwuh beverage before gastrointestinal digestion, and B is total phenolic content or antioxidant capacity from bioaccessible fraction.

Statistical Aanalyzed

The Minitab version 20.3 software was used to evaluate the data using One-Way analyzed of variance (ANOVA). Tukey's test was carried out to verify the significance difference between the means at $p<0.05$. All data were presented as average ± SD (n=2).

Results

The Composition of the Wedang Uwuh

This study used commercial wedang uwuh product which has the highest sales level and good consumer acceptance reviews. The ingredients of wedang uwuh consist of dried emprit ginger

(*Zingiber officinale var amarum*), sappan wood (*Caesalpinia sappan* L), clove leaves and stems (*Syzygium aromaticum*), cinnamon leaves (*Cinnamomum zeylanicum*), and nutmeg leaves (*Myristica fragrans*) as shown in Figure 1 with the formulation as shown in Table 1.

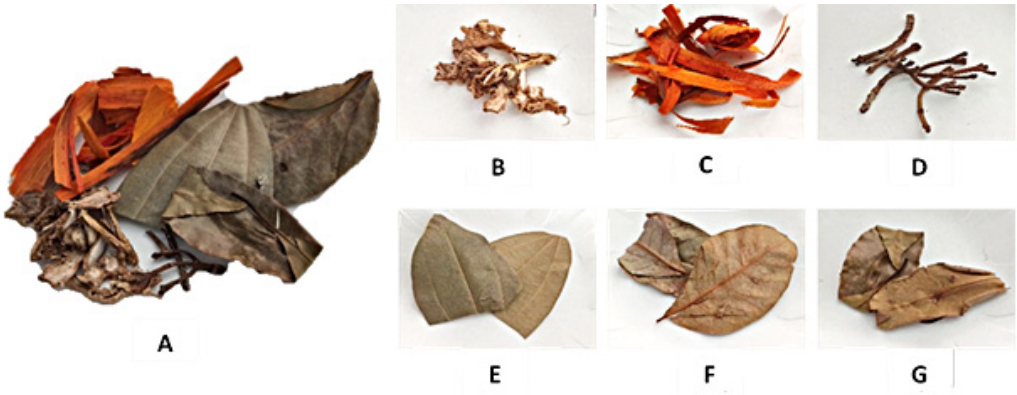

Fig. 1: The ingredients of wedang uwuh. Ingredients in one serving size (A); and dried herbs, i.e., gingers (B); sappanwood (C); clove stems (D); cinnamon leaves (E); nutmeg leaves (F); and clove leaves (G)

Phenolic Content and Antioxidants Capacity Prior to D igestion

Table 3 shows the total phenolic content and antioxidant capacity of the wedang uwuh beverage before going through digestion at different boiling times (5, 10 and 15 minutes). In general, the results of the analysis showed that the total phenolic content and antioxidant capacity of the wedang uwuh beverage increased significantly ($p<0.05$) according to the boiling time. Boiling time for 15 minutes produced the highest total phenolic content of 65.48 ± 0.6 mg GAE/serving and the highest antioxidant capacity of 62.25 ± 0.1 mg TE/serving (FRAP) and 96.17 ± 0.1 mg TE/serving (DPPH). However, the ABTS method produced the highest antioxidant capacity at 10 minutes of boiling and was not significantly different ($p>0.05$) from 15 minutes of boiling.

Table 3: Total phenolic content dan antioxidant capacity wedang uwuh beverage prior to digestion.

Sample	Total phenolic content (mg GAE/ serving)	Antioxidant capacity (mg TE/serving)		
		FRAP	DPPH	ABTS
R5	54.49±0.8^{c}	56.13±0.1^{c}	84.76±0.3^{c}	115.36±0.3^{b}
R10	61.84±0.6^{b}	61.80±0.1^{b}	95.30±0.2^{b}	123.50±1.9^{a}
R15	65.48±0.6^{a}	62.25±0.1^{a}	96.17±0.1^{a}	123.39±1.1^{a}

Total phenolic content and antioxidant capacity of wedang uwuh beverage (R) before digestion at different boiling times (5,10,15 min). Significantly different ($p<0.05$) measurement results between boiling time variables are represented by differences in superscript letters in the same column. Data values are expressed in mean±SD (n=2).

Phenolic Content and Antioxidants Capacity After Digestion

Table 4 and Figure 2 show the total phenolic content and antioxidant capacity of wedang uwuh beverage after *in vitro* gastrointestinal digestion. In Figure 2, after gastric digestion there was a significant reduction in total phenolic content and antioxidant capacity ($p<0.05$). In this phase, the boiling time

still showed a significant effect ($p<0.05$) on the total phenolic content and antioxidant capacity in the wedang uwuh beverage. The data in Table 5 shows that boiling for 15 minutes produced the highest total phenolic content of 45.85±1.7 mg GAE/serving and the highest antioxidant capacity of 57.25±0.4 mg TE/serving (FRAP) and 76.27±0.9 mg TE/serving (DPPH), except the ABTS method showed no significant difference in antioxidant capacity ($p>0.05$) at different boiling times.

Table 4: Total phenolic content dan antioxidant capacity wedang uwuh beverage during the digestion process

Sample (mg GAE/ serving)	Total phenolic content (mg TE/serving)	Antioxidant capacity		
		FRAP	DPPH	ABTS
Gastric digestion				
R5	28.80±0.1^{c}	53.30±0.4^{b}	33.24±1.3^{c}	113.08±0.1^{a}
R10	37.26±1.5^{b}	55.47±0.8^{ab}	53.38±1.8^{b}	111.67±0.6^{a}
R15	45.85±1.7^{a}	57.25±0.4^{a}	76.27±0.9^{a}	108.85±0.9^{b}
Intestinal digestion				
R5	18.19±1.1^{a}	22.91±1.9^{a}	24.83±0.8^{a}	107.87±0.8^{a}
R10	18.43±0.8^{a}	18.26±0.0^{a}	22.21±2.7^{ab}	106.90±1.5^{a}
R15	19.39±0.3^{a}	20.80±0.4^{a}	17.62±0.8^{b}	106.03±0.9^{a}
Supernatant from intestinal digestion				
R5	17.58±1.1^{a}	20.90±1.8^{a}	21.93±1.3^{a}	96.26±0.9^{b}
R10	17.79±0.5^{a}	17.30±1.0^{a}	20.59±1.4^{ab}	102.56±1.5^{a}
R15	18.35±0.6^{a}	19.26±0.0^{a}	16.00±1.0^{b}	100.60±1.1^{ab}

Total phenolic content and antioxidant capacity of wedang uwuh beverage (R) during gastrointestinal digestion at different boiling times (5,10,15 min). Significantly different ($p<0.05$) measurement results between boiling time variables are represented by differences in superscript letters in the same column. Data values are expressed in mean±SD (n=2).

After passing through the intestinal digestion there was a greater decrease in total phenolic content and antioxidant capacity, and the length of boiling time had no significant effect ($p>0.05$) on the value of total phenolic content and antioxidant capacity. The bioaccessible fraction is described by the supernatant resulting from the intestinal digestion which is the soluble fraction resulting from the *in vitro* gastrointestinal digestion. In general, boiling time for 5 minutes showed total phenolic content and antioxidant capacity which was not significantly different ($p>0.05$) from the longer boiling time. The values of total phenolic content and antioxidant capacity in boiling 5 minutes after digestion were 17.58±1.1 mg GAE/serving (total phenolic content), 20.90±1.8 mg TE/serving (FRAP), and 21.93±1.3 mg TE/serving (DPPH). The antioxidant capacity using ABTS method showed higher results than other methods of 96.26 ± 0.9 - 100.60 ± 1.1 mg TE/serving.

Antioxidant Bioaccessibility of Wedang Uwuh Beverage

Table 6 shows the bioaccessibility index of antioxidants and phenolics in wedang uwuh beverage after *in vitro* gastrointestinal digestion. Boiling time of 5 minutes showed phenolic and antioxidant bioaccessibility index of 32.25± 1.5 % (total phenolic content), 37.25 ± 3.2 % (FRAP), 25.88 ± 1.6 % (DPPH), and 83.45 ± 1.0 (ABTS) that were not significantly different with longer boiling times. In addition, the data in Table 5 shows that boiling for more than 5 minutes produces a lower bioaccessible fraction of phenolic compounds and antioxidants.

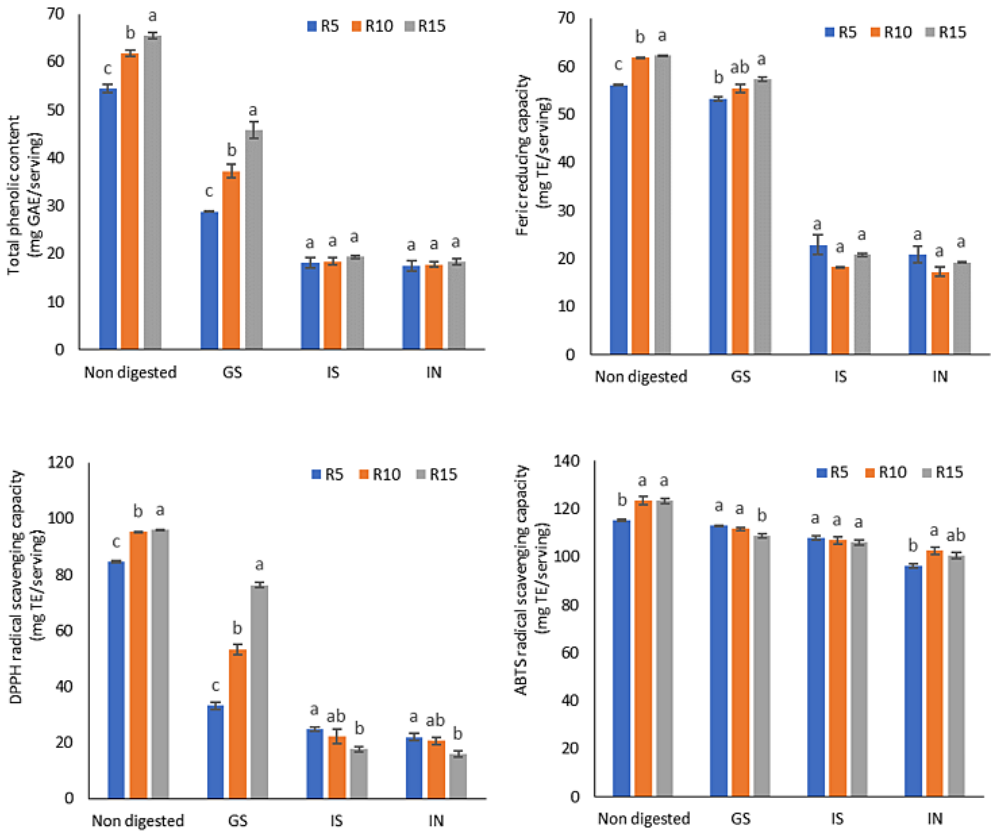

Fig. 2: Total phenolic content and antioxidant capacity of wedang uwuh beverage (R). Wedang uwuh beverage showed a decrease in total phenolic content and antioxidant capacity at the gastric digestion stage (GS), and the intestinal digestion stage (IS) at different boiling times (5,10,15 min). IN is described as bioaccessible fraction after intestinal digestion stage.

Table 6: Bioaccessibility index wedang uwuh beverage after gastrointestinal digestion

Sample	Bioaccessibility index (%)			
	TPC	FRAP	DPPH	ABTS
R5	32.25±1.5^{a}	37.25±3.2^{a}	25.88±1.6^{a}	83.45±1.0^{a}
R10	28.77±0.6^{ab}	27.98±1.7^{b}	21.61±1.4^{ab}	83.04±0.0^{a}
R15	28.03±0.7^{b}	30.94±0.0^{ab}	16.63±1.1^{b}	81.53±0.2^{a}

Bioaccessibility index of wedang uwuh extracts (R) at different boiling times (5, 10, 15 min). Significantly different ($p<0.05$) measurement results between boiling time variables are represented by differences in superscript letters in the same column. Data values are expressed in mean±SD (n=2).

Discussion

Wedang uwuh is a traditional beverage originating from Yogyakarta, Indonesia. This beverage is reddish color with a pungent and warm taste. The red color comes from the brazilein compound which is extracted from the sappan wood. Meanwhile, the flavor and warm taste come from other ingredients such as ginger, cloves, nutmeg and cinnamon leaves.[5] This study used wedang uwuh with the composition as in Table 1 without using rock sugar. The addition of sugar can affect the test results for total phenolic content because sugar can reduce the folin-ciocalteu reagent, so that it can result in false positives.[28] In addition, the added sugar in beverage products has an effects on the level of phenolic compounds and antioxidants bioaccessibility. The addition of sugar can increase the stability of the phenolic components to digestive conditions.[29] In cinnamon drinks, sugar causes an increase in the bioaccessibility of polyphenols because it can reduce the formation of pepsin-tannin precipitates.[30] However, research on grape juice stated that the bioavailability of quercetin decreased in samples with added sugar, because the sugar moiety attached to quercetin interfered with the absorption of quercetin.[31]

Wedang uwuh is the best source of phenolic and antioxidant compounds, which are obtained from the ingredients. Dry emprit ginger is the ingredient with the highest percentage. Dried ginger contains gingerol and shogaol compounds as bioactive compounds, which have high antioxidant activity.[32] The second most composition is sappan wood. Sappan wood contains the active compound brazilin which forms a red color in the aqueous extract when oxidized to brazilein, due to the release of H+ ions from the hydroxyl group of the benzene ring.[7,33] Scientific studies show that brazilein has activity as an antioxidant.[8] Other ingredients in wedang uwuh are clove stem and leaves. Cloves contain eugenol as bioactive compound which has strong antioxidant activity in reducing ferric ions and scavenging free radicals.[9] In addition, nutmeg and cinnamon leaves are also good sources of phenolic compounds and antioxidants.[5] Cinnamon leaves contain cinnamaldehyde and tannins as the major phenolic compounds.[26] Eugenol is also abundantly present in the cinnamon and nutmeg leaves.[34,35] Eugenol is found in the four ingredients of wedang uwuh that are clove (stems and leaves), cinnamon leaves, and nutmeg leaves. The antioxidant capacity of wedang uwuh beverage is the result of the interaction of the antioxidant compounds from the constituent ingredients.

Food processing can also affect antioxidant capacity both before and after digestion. Previous studies have shown that wedang uwuh beverage with a longer boiling time produces greater total phenolic content and antioxidant activity.[11] This is in line with the results of this study which showed that the longer boiling time significantly ($p<0.05$) increased the total phenolic content as well as antioxidant capacity in wedang uwuh beverages. Boiling for 15 minutes resulted in the highest total phenolic content and antioxidant capacity in the wedang uwuh beverages, as shown in Table 2. The longer boiling time also increased epicatechin and catechin in hawthorn berry extract.[36] In addition, the total phenolic content in the infusion of black tea and white tea also increased up to 15 minutes of brewing time.[37,38] This proves that the longer boiling time will increase the antioxidant capacity because the longer the contact time of the material with the solvent will increase the amount of dissolved antioxidant compounds. The increase in antioxidant capacity and total phenolic content occurs because the heating process during boiling can damage the cell walls and cause changes in cellular structure, which results in the dissolving of phenolic and antioxidant components into the solvent.[36]

A food ingredient must pass through the digestive system and be able to survive with the changing conditions in digestion, such as digestive enzyme activity and pH, before it can be absorbed and utilized by the body.[39] Wedang uwuh beverage contains bioactive compounds as a source of antioxidants in the body.[5] The results showed that the total phenolic and antioxidant capacities of the wedang uwuh beverage decreased significantly ($p<0.05$) after the simulated gastric digestion process. The decrease in total phenolic after gastric digestion was also reported to occur in infusions of moringa leaves and persimmon leaves.[27,40] This occurs because pH conditions and digestive enzyme activity can cause changes in chemical structure and degradation of several phenolic compounds.[36,41] Decreases in total phenolic content and antioxidant capacity may occur because some phenolic compounds are unstable and degraded during gastric digestion.

The acidic pH in the gastric environment causes hydrolysis and transformation of the chemical structure of phenolic compounds and fat-soluble vitamins.[18] Although there was a decrease in the total phenolic and capacity of antioxidant of the wedang uwuh beverage after gastric digestion, boiling time for 15 minutes still showed a significant effect ($p<0.05$) on the total phenolic and capacity of antioxidant. This may be due to the amount of initial components extracted during sample preparation.

After the gastric digestion phase, food is transferred to the intestinal digestive phase and then absorbed and used by the body. The alkaline environment in intestinal digestion may cause the phenolic chemical structure to be unstable.[42] Data from the analysis showed that there was a sharper decrease compared to the gastric digestion phase in the value of total phenolic content and antioxidant capacity in the wedang uwuh beverage after passing through the intestinal digestion, as Figure 2. The boiling process for 15 minutes showed the highest reduction in total phenolic content and antioxidant capacity of 70% and 14 – 82%, respectively (data not shown). The heat processing may increase in extractability of phenolic compounds, but it also destroys some thermo-labile compounds.[36] This causes phenolic compounds and antioxidants to become more susceptible and easily damaged by different pH levels during gastric and intestinal digestion. Similar results were also reported for persimmon leaves infusion,[27] green tea infusions,15 and hawthorn berry decoction.[36] that there was a decrease in total phenolic content and antioxidant capacity after *in vitro* digestion. Conversely, an increase in total phenolic content and antioxidant capacity after *in vitro* digestion was reported in jackfruit extract,[21] fruit juice,[28] and vegetable juices.[20] This occurs because the pH conditions and digestive enzyme activity causes the food matrix to decompose thereby increasing the release of phenolic compounds.[27,43] A decrease or increase in total phenolic and antioxidant capacity after digestion indicates a chemical change in phenolic and antioxidant compounds during the gastrointestinal digestion process due to pH conditions and digestive enzyme activity.[36,43]

The decrease in total phenolic content and antioxidant capacity in the wedang uwuh beverage after going through the *in vitro* gastrointestinal digestion may be due to a modification of the chemical structure from the extracted ingredients. Previous research reported that the concentration of gingerol in ginger extract decreased after passing through the *in vitro* gastrointestinal digestion.[44] The cinnamon beverage has lower total phenolic content and antioxidant capacity after gastric-pancreatic digestion due to the precipitation of tannins, which interacts with pepsin in the gastric phase.[30] Scientific research reported that brazilin and phenolic compounds in sappan wood extract showed the highest stability in acid environment, but easily oxidized by adding NAOH (0.1 N) which causes deprotonation of the -OH group and changes in the structure of the brazilein molecule.[7,45] Additionally, clove and nutmeg were reported to be stable during digestion.[46]

The boiling process for 15 minutes resulted in the highest total phenolic and antioxidant capacity before consumed, but after the *in vitro* gastrointestinal digestion process there was a significant decrease ($p<0.05$). After passing through the gastric and intestinal digestion, the boiling time did not significantly ($p>0.05$) affect the bioaccessible fraction of phenolic and antioxidant compounds in wedang uwuh beverage. The bioaccessible fraction was calculated from the supernatant resulting from the centrifugation of the digestive solution in intestine. The supernatant describes the fraction that can be digested and contains bioactive components that are able to survive digestive conditions so that they can be absorbed by the body.[22] The boiling process for 5 minutes resulted in a higher number of bioaccessible fractions and antioxidant bioaccessibility index but not significantly different ($p>0.05$) from the longer boiling time. This shows that boiling time which is longer than 5 minutes causes greater damage to phenolic and antioxidants compounds so that they are more susceptible to damage during the digestion process. The results of previous studies on ready-to-drink wedang uwuh showed that there was thermal degradation of antioxidant activity at higher pasteurization temperatures.[47] Similar results were also reported that the lowest bioaccessibility of phenolic compounds occurred in fruit drink products with heat treatment compared to non-thermal methods.48 In hawthorn berry fruit extract, there was a decrease in total phenolic bioaccessibility and antioxidant activity in the boiling process for

more than 15 minutes.[36] The heating process can affect the stability of phenolic compounds before and after digestion, thereby reducing their antioxidant activity.[15,49] Furthermore, the heating process can change the chemical structure of phenolic compounds causing an increase or decrease in bioaccessibility by promoting the release of phenolics from the food matrix, chemical structural degradation, or polymerization.[48]

The ABTS test resulted in the highest bioaccessibility index and antioxidant capacity compared to the FRAP and DPPH methods. This can occur due to differences in the mechanism of the test method.[22] The digestion process followed by a structural transformation of phenolic compounds and antioxidants which causes interactions between sample constituents, thus influencing their detection using similar or more sensitive methods.[50] In addition, the results of other studies have reported that the test method with ABTS does indeed show better sensitivity than DPPH for food samples that are hydrophilic, lipophilic, and contain high pigment components.[51] From the analysis it can be concluded that boiling for 5 minutes is the best way for preparing wedang uwuh beverage, with antioxidant bioaccessibility index of 37.25% (FRAP), 25.88% (DPPH) and 83.45% (ABTS). Previous studies reported variations in the bioaccessibility of phenolic and antioxidant compounds in different beverages, i.e. 18.0 – 25.9% for the phenolic compounds bioaccessibility of fruit juice drinks,[48] 24.6% for the antioxidants bioaccessibility of persimmon leaves infusion[27] and 10% for bioactivity of mono-caffeoylquinic acids from J. glutinosa infusion.[22] In addition, differences in the chemical structure of phenolic and antioxidant compounds, as well as the food matrix, may also affect behavior and stability under *in vitro* gastrointestinal digestion.[18,21]

Conclusion

This study concluded that phenolic and antioxidant compounds in wedang uwuh beverages were sensitive to the gastrointestinal environment. Before gastrointestinal digestion, boiling for 15 minutes produced the highest total phenolic content and antioxidant capacity in the wedang uwuh beverage. However, there was a decrease in the total phenolic content and antioxidant capacity in the wedang uwuh beverages after the *in vitro* gastrointestinal digestion due to digestive conditions such as pH and enzyme activity. A boiling time of 5 minutes gave the highest total phenolic content, antioxidant capacity and antioxidant bioaccessibility index in the wedang uwuh beverages after the *in vitro* gastrointestinal digestion. Therefore, 5 minutes of boiling time is recommended as the best way to prepare wedang uwuh beverage. Further research is envisaged to investigate the composition changes of phenolic and antioxidant compounds of wedang uwuh during the digestion process.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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