The effect of light and temperature in the traditional "Wedang Uwuh" ready-to-drink (RTD) beverages on color stability and brazilin content during storage

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Abstract. Wedang uwuh, a traditional drink from Yogyakarta, has potential as a functional beverage due to its antioxidant-rich ingredients like sappan wood, ginger, and cinnamon leaves. A key challenge in developing ready-to- drink (RTD) wedang uwuh is maintaining the red color, derived from brazilin in sappan wood, which also offers strong antioxidant benefits. This study aims to determine the effects of light and temperature on wedang uwuh ready to drink on color stability and brazilin content during storage. Samples consisted of control (C) which is a commercial wedang uwuh on the market without treatment and wedang uwuh treated with water blanching (1 minute, 85°C) and cabinet drying (12 hours, 55°C). The dried powder samples were then brewed at 85°C for 20 minutes, filtered and put in clear and brown glass bottles to store at room temperature to determine the effect of light. As for temperature, the samples were stored at room temperature (30°C), cold temperature (5°C) and Air Condition (AC) temperature (16°C). The results showed that light and temperature had an effect on color stability and brazilin content during 12-day storage. The a* value which shows the degree of red color in wedang uwuh RTD drinks during storage shows a greater decrease in clear bottles of 5.29 and 4.33 in control samples and samples with treatment than in brown bottles of 4.33 and 0.78 in control samples and samples with treatment. Brazilin content also decreased more in clear bottle storage. While at temperature storage, the best results were obtained at cold temperature storage (5°C) on color stability and brazilin content during 12 days storage.

1 Introduction

It has been known that spices have many health benefits due to the content of active compounds that have functional value such as ginger which contains gingerol, zingerone, shogaols, β-phellandrene, camphene, and cineol as a source of antioxidants and flavour. [1].

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Ginger is commonly used in traditional dishes and beverages in Indonesia, one of which is wedang uwuh. Wedang uwuh originates from Imogiri sub-district, Bantul regency, Yogyakarta. This traditional ancestral drink has received worldwide recognition for its health benefits. The main ingredients are ginger and sappan wood [2]. According to an interview with one of the colourists of Imogiri Bantul, the original wedang uwuh consists of ginger, sappan wood, clove leaves, nutmeg leaves, cinnamon leaves, and rock sugar [3]. Besides ginger, sappan wood is the main ingredient in wedang uwuh, which gives the drink a more attractive appearance because it has red pigments that give the drink its colour.

Brazilin in secang wood is a red colour producing compound that is slightly soluble in cold water and easily soluble in hot water. Secang contains various phenolic components including xanthones, coumarins, chalcones, flavones, homoisoflavonoids, and brazilin which are widely used as antioxidants, antibacterial, anti-inflammatory, antiaging, hypoglycaemia, hepatoprotectors, and anti-acne [4].

Caesalpinia sappan L. is distributed throughout Southeast Asia and dried Caesalpinia sappan L. heartwood (CSH) has been used in Thai traditional medicine, for example to treat anemia, skin infections, tuberculosis, and diarrhea [5]. Many researchers report that the chemical compounds of CSH are phenolic compounds; for example, brazilin, protosappanin, chalcone, etc. The main bioactive natural compound of CSH is brazilin. Brazilin is a weakly colored product and has also been used in the food, beverage, cosmetic, and pharmaceutical industries [6]. Brazilin is a weakly colored product and has also been used in the food, beverage, cosmetic, and pharmaceutical industries [7].

Brazilin has been reported to have a wide range of biological activities including antibacterial, anti-inflammatory, anti-photoaging, hypoglycemic, vasorelaxant, anti-allergic, anti-acne, and antioxidant activities. In addition, CSH water extract has many beneficial properties, including having no unique flavor, being tasteless, and inexpensive. Brazilin consists of two aromatic rings, namely one pyrone ring and one five-membered ring. However, the hydroxyl group on the brazilin structure is easily oxidized and can change into a carbonyl group, causing the transformation of the structure into a brazilein compound. Brazilein has been used as a natural dye to produce red color in traditional foods and herbal drinks in many Asian countries [7]. Brewed wedang uwuh has the lowest IC50 value compared to other variants of wedang uwuh which is 9195,40 ppm. This means that the brewed wedang uwuh has the best antioxidant activity compared to the original, dipped, instant, and syrup wedang uwuh [8].

Wedang uwuh drinks have currently been developed in various forms of presentation such as concoction, brewed, instant, dipped and instant, but are still very limited in the form of "ready-to-drink (RTD)" due to their stability during storage, whereas wedang uwuh ready to drink or "ready to drink" is an alternative in making contemporary products that are easy to serve, attractive and practical. The functional properties of ready-to-drink wedang uwuh need to be maintained with proper production, packaging and storage processes [9]. The functionality of several ingredients in wedang uwuh drinks in their function to prevent and minimize the occurrence of degenerative diseases, namely antioxidants, lower cholesterol, prevent osteoporosis anti-diarrhea, anti-cancer. Herbal drinks have now been widely developed whose ingredients are not only based on one or two ingredients [10].

Color change can be influenced by storage temperature factors. Color can be affected by pigment content, pH, temperature, oxygen, and light. pH affects the color changes that occur [3]. Glass bottles are the best packaging to maintain the stability of the sour turmeric drink [11]. In clear (bright) bottles, direct contact with light causes electron conjugation due to the energy derived from ultraviolet light so that the color becomes brownish red and the degradation of bright yellow (initial color) increases, with a color change of up to 97%. While in the dark bottle the color is increasingly degraded by light marked by the color increasingly pale. In dark bottles there is no absorption of energy from light, so there is no conjugation of

electrons and the color becomes clear yellow, the decrease in intensity changes by 34%. This shows that the type of storage place affects the color. Brown glass bottles tend to block ultraviolet rays that interact with pigments. So that the level of pigment damage is reduced compared to clear glass bottles [12].

The color change comes from sappan wood as an ingredient that produces brazillin pigment. Brazillin in sappan wood is a pigment that produces a red color that dissolves easily with the addition of hot water, so the boiling process optimizes pigment extraction and produces a fresh red color in wedang uwuh [13]. pH affects the change in color intensity that occurs. The color change during the storage process is caused by the brazilein pigment of sappan wood. Acidic conditions during storage cause color fading in wedang uwuh. This result is in accordance with research on brazilien pigment extract at pH 6-7 or neutral gives the appearance of a concentrated red color, while pH 5 shows easy red or brownish red [14].

Since research on the stability of wedang uwuh drinks during storage is still limited, this study was conducted to determine the effect of light and temperature during storage on color stability and brazilin content in wedang uwuh RTD drinks. The results of this study can be a recommendation for producers of traditional wedang uwuh drinks in the development of ready to drink drinks and support Sustainable Development Goal's (SDG's) No. 3, namely good health and well-being.

2 Material and Methods

2.1. Materials and tools

Materials used such as sappan wood obtained from the forest in Gunungkidul, ginger emprit, cinnamon leaves, nutmeg leaves, clove leaves and clove stalks from farmers in Kulonprogo Yogyakarta. Other materials used are silica gel, LeMineral water, aluminium foil, sodium benzoate, water injection, acetonitrile, distilled water, filter paper, tissue, label paper.

The tools used in the research are cabinet dryer, refrigerator, storage box, grinder, sieve, container, zip lock plastic packaging, pot, electric stove, brown and transparent glass bottles, funnel, clear vial bottle (documentation), 2 ml HPLC vial bottle, HPLC syringe filter nylon 0.45 µm 13 mm, glass funnel, 100 ml beaker glass, 1 ml measuring pipette, green propipette, dropper pipette, spray bottle, vacuum device, syringe (for HPLC), a set of HPLC tools, pH meter, spectrophotometer, and Minolta CR-400 chromameter.

2.2 Treatment of raw material samples

The main ingredients used in making ready-to-drink wedang uwuh samples are ginger, nutmeg leaves, cinnamon leaves, clove leaves, clove handles, and sappan wood obtained from CV. Progress Jogja [3]. The wedang uwuh drink samples were made from a mixture of sappan wood, ginger, nutmeg leaves, clove leaves, cinnamon leaves, and clove stalks in a certain ratio. There were 2 samples, namely a control sample of commercial wedang uwuh on the market with ingredients used without special treatment, namely fresh raw materials dried by sunlight and a sample of wedang uwuh ingredients that were given preliminary treatment, namely water blanching for 1 minute at 85 C and drying with a cabinet dryer at 55 C for 12 hours.

2.3. Sample preparation

Ingredients such as sappan wood, ginger, cinnamon leaves, clove leaves, nutmeg leaves, and clove handles after drying are finely ground and then formulated in a ratio of 9:5:1:1:1:1. To make ready to drink wedang uwuh, each control sample and treated sample was brewed with

boiling water at 85ºC for 20 minutes. The steeping water was then filtered to separate the residue from the powder material. After filtering, the samples were poured into brown and transparent bottles for storage and analysis.

2.4. Storage treatment

The effect of light and temperature on red colour stability and brazilin content will be tested during 12 days storage. To determine the effect of light during storage, clear glass bottles and brown bottles are used to store the samples for 12 days. Meanwhile, to determine the effect of temperature, the samples were treated with storage at room temperature (30ºC), cold temperature of 5ºC and AC (Air Condition) temperature of 16ºC. These temperatures were chosen because they are representative of product storage in the market. Storage was carried out for 12 days with observation time every 0, 3, 6, 9, and 12 days for colour testing and every day 0, 6 and 12 for testing brazilin content.

2.5. Procedure for brazilin analysis

Samples with various treatments were put in 2 ml HPLC vials and then tested with HPLC equipment. HPLC testing was also carried out every 3 days for 12 days.

Determination of brazilin content, analysis of brazilin was performed using a high-performance liquid chromatograph (Waters, Massachusetts, USA) consisting of a quaternary pump, on-line degasser, autosampler, and photodiode array detector. The detector wavelength was set at 286 nm. Separation was performed using a Reliant C18 analytical column (150 mm 4.6 mm, 5 mm). The mobile phase consisted of water (A) and acetonitrile (B) with the following gradient: 0.00-2.00 min, 15% B; 2.00-5.00 min, 15-25% B; 5.00-9.00 min, 25% B; 9.00-9.10 min, 25-100% B; 9.10-13.00 min, 100% B; 13.00-13.10 min, 100-15% B; 13.10-17.00 min, 15% B) with a flow rate of 1.0 mL min-1 and run at 40°C. Ten grams and five grams of sample were dissolved in 50 mL and 25 mL MeOH, respectively, followed by sonication extraction at 30°C for 30 minutes. The extraction was repeated three times. The extracted solvent was evaporated using a rotary evaporator. Next, ten milligrams of dried extract was dissolved in 1000 mL MeOH. The solution was then diluted 10 and 5 times for pure and mixed herbal drink samples. The diluted solution was filtered with 0.22 µm PTFE, and 5 µl samples were then injected into the HPLC.

2.6. Colour testing

Fifteen milligrams of sample was brewed in 150 mL of water at 85°C for 15 minutes. The sample was then centrifuged at 2330 g for 15 minutes. The liquid was transferred into a petri dish and inserted into a chromameter (Minolta CR-400, Osaka Japan). The results were displayed as numbers through L* (brightness ranges from 0-100), a* (from green to red) and b* (from blue to yellow) values. The brightness notation is expressed as a brightness parameter with a value between 0-100, ranging from dark to bright. The redness notation is expressed as the colour parameter of green and red, values from (-80) ± (+100) from green to red and the yellowish notation is expressed as the colour from blue to yellow with a value range from (-70) ± (+70). Statistical analysis was performed with SPPS Statistic 19. Analysis was carried out with One Way Anova and followed by a significant difference test with DMRT (Duncan Multiple Range Test) with a significance level of 0.05%.

2.7. Data analysis

Statistical analysis was performed with SPPS Statistic 19. Analysis was carried out with One Way Anova and followed by a significant difference test with DMRT (Duncan Multiple Range Test) with a significance level of 0.05%.

3 Results and Discussion

3.1. Effect of Light on red color (a*)

Red color stability and brazilin content in wedang uwuh can be affected by light exposure during storage (Table 1). Research on the effect of light and packaging type on pigment stability is very important to understand the best way to maintain the quality of wedang uwuh. in ready to drink beverage products, packaging is also one aspect of product safety and durability as well as attractiveness to consumers. bottle packaging made of plastic, glass with clear or colored colors will have an influence during storage. in this study, clear bottles and brown bottles were used during storage.

Table 1. Red color (a*) during storage

Time (day)	CB	CC	DB	DC
0	14.45	11.26	19.28	17.73
3	16.34	13.86	17.43	15.86
6	11.05	10.22	13.10	15.08
9	10.62	10.06	11.79	7.96
12	12.80	12.37	13.13	8.04

*Description: CB (No treatment, clear bottle), CC (No treatment, brown bottle), DB (Treatment, clear bottle) and DC (Treatment, brown bottle)

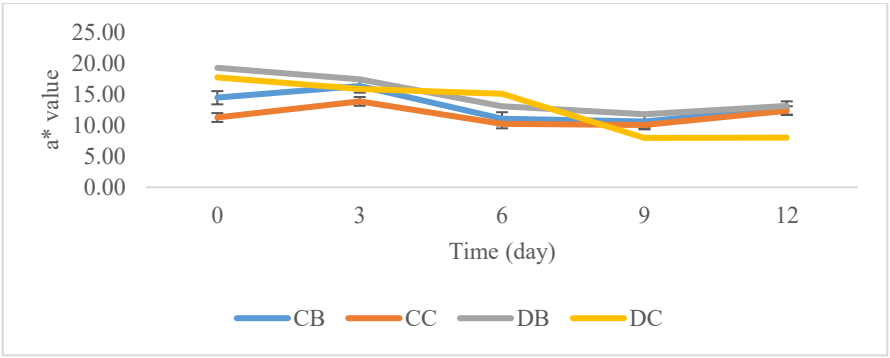

Fig. 1. Effect of light on the red color (a*) of wedang uwuh RTD during storage

According to the color test results of samples CB, CC, DB, and DC, samples CB (No treatment, clear bottle) and CC (No treatment, brown bottle) are controlled and without blanching treatment. While samples DB (Treatment, clear bottle) and DC (Treatment, brown bottle) as samples with blanching treatment. When viewed from samples with different bottles used on day 0, sample CB has a higher redness (a) color indicator than sample CC and sample DB redness (a*) indicator is higher than sample DC. This shows that storage with clear bottles increases redness (a*) color than brown bottles. However, on storage day 6, sample CB decreased by 5.29 while sample CC decreased by 3.64 and sample DB decreased by 4.33 while sample DC decreased by 0.78. Thus, in general, storage with clear and brown

bottles decreased in color during the storage period. However, samples stored with clear bottles experienced a greater decrease compared to brown bottles [15].

The redness color indicator (a) of samples CB and CC on storage day 3 increased then the next storage day both decreased (Figure 1). This causes the color of wedang uwuh to change from fresh red to dark brownish red. Another color quality measurement parameter is redness (a*), the a* value shows the degree of red color in the sample. The longer the storage time shows a decrease in the degree of red color [9].

The wedang uwuh samples without blanching treatment CB and CC when compared to the blanching treatment samples DB and DC, in general, the untreated samples had smaller a* values than the blanching treatment samples. In accordance with wedang uwuh ready to drink research, there was a decrease in redness color (a*) in non-blanching wedang uwuh ready to drink. This result is in accordance with the effect of temperature and storage time which the longer the time causes a decrease in redness color (a*) [3]. Blanching serves to deactivate enzymes, so that the resulting color is better or more intense. In addition, blanching is also able to prevent or inhibit unwanted color changes, improve flavor or aroma [16]. The decrease in a value is also in line with the length of storage time, samples from day 0 to day 12 a* value also decreases. This shows that the longer the storage time, the lower the degree of red color [9].

3.2. Effect of Temperature on color (a*)

The wedang uwuh samples without treatment G30, G5, and G16 when compared to the blanching treatment wedang uwuh samples H30, H5, and H16 in general, the blanching treatment samples have a higher a value than the untreated samples (Table 2). This shows that blanching treatment can reduce the decrease in color a* in the wedang uwuh sample. According to wedang uwuh ready to drink research, there is a decrease in redness color (a*) in non-blanching wedang uwuh ready to drink. This result is in accordance with the effect of temperature and storage time, which the longer the time causes a decrease in redness color (a) [3]. Blanching serves to deactivate enzymes, so that the resulting color is better or concentrated. In addition, blanching can also prevent or inhibit unwanted color changes, improve flavor or aroma [16]. The decrease in a value is also influenced by the length of storage time, the longer the storage time shows a decrease in the degree of red color [9].

Table 2. Red color (a*) during storage

Time (day)	G30	G5	G16	H30	H5	H16
0	13.52	13.52	13.52	17.10	17.10	17.10
3	12.11	15.70	13.79	9.92	18.43	10.30
6	13.97	16.39	10.62	11.82	11.50	11.90
9	14.63	11.98	12.28	13.72	12.19	13.92
12	15.56	14.09	11.93	11.35	17.39	17.12

*Description: G30 (Control, no treatment, temperature 30), G5 (Control, no treatment, temperature 5), G16 (Control, no treatment, temperature 16), H30 (Treatment, temperature 30), and H5 (Treatment, temperature 5), and H16 (Treatment, temperature 16)

According to the untreated samples G30, G5, and G16, sample G5, which was stored at 5°C on day 3, had the highest a-value compared to sample G30 stored at 30ºC and sample G16 at 16°C. In order, the samples that have the highest a-value to the lowest are G5, G16, and G30. These results are consistent with the a* value at low temperatures lasting longer than at high temperatures. [3]. This also occurs in samples with blanching treatment, in order

the samples that have the highest a-values are samples H5, H16, and H30, which are the lowest (Figure 2).

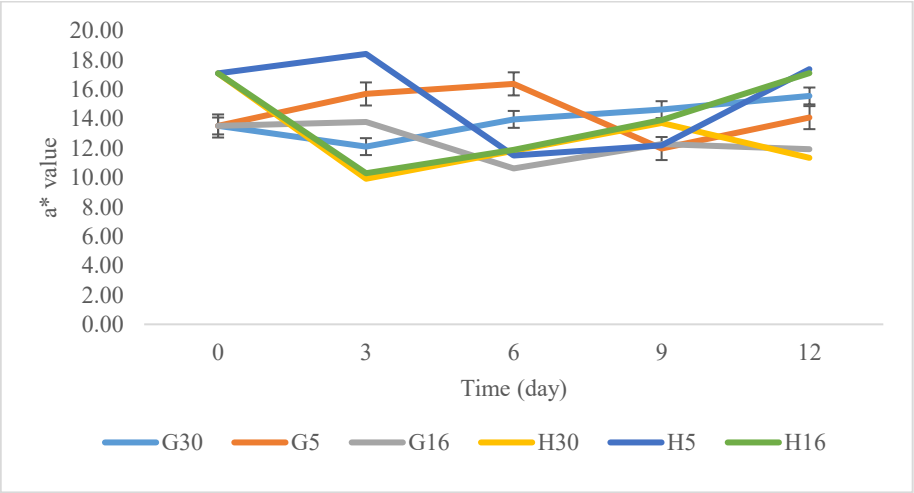

Fig. 2. Effect of temperature on the red color (a*) of wedang uwuh RTD during storage.

3.3. Effect of Light on brazilin content

According to Table 3. It can be seen that during storage there was a decrease in brazilin content in all samples. The decrease in brazilin content in the control sample was 8% in the clear bottle treatment and 7% in the brown bottle treatment, while in the sample with blanching treatment there was a decrease of 7% in the clear bottle and 6% in the brown bottle. Although the statistical results showed no significant difference between the treatment of using clear bottles and brown bottles, the decrease in brazilin content in brown bottles was lower than in clear bottles. Brazilin is easily oxidized and loses 1 hydrogen atom when exposed to light and oxygen [17]. Therefore, the clear bottle is exposed to higher intensity light than the brown bottle which causes a higher decrease in brazilin content during storage due to photooxidation.

Table 3. Brazilin content (%) during storage

Time (day)	Brazilin content (%)			
	CB	CC	DB	DC
0	0.27 ± 0.01cd	0.29 ± 0.84c	0.23 ± 0.04abc	0.23 ± 0.04abc
6	0.22 ± 0.04abc	0.29 ± 0.01c	0.20 ± 0.02ab	0.21 ± 0.01abc
12	0.19 ± 0.01ab	0.22 ± 0.04b	0.16 ± 0.02a	0.17 ± 0.03a

*Description: Average ± Standard Deviation

Blanching treatment during preparation showed an effect on lowering brazilin content compared to the control. This happens because blanching treatment can inactivate enzymes that can cause brazilin degradation. Enzymes such as polyphenol oxidase and peroxidase can damage pigments and phenolic compounds during storage and processing. By heating the material at a certain temperature for a short time (blanching and cabinet drying), these enzymes are inactivated resulting in a more stable brazilin content. The heat treatment applied during blanching can reduce the rate of oxidation reactions that can affect the brazilin content. By reducing oxidation, the color stability and antioxidant activity of brazilin can be maintained. Sappan wood has a red color called brazilin which is found in the deepest part

of sappan wood [18]. The blanching process can reduce or inactivate enzymes such as peroxidase (POD) and polyphenol oxidase (PPO) that contribute to negative effects such as undesirable taste, odor, color, texture, and loss of nutrients and phytochemicals [19]. Brazilin on oxidation produces a stronger red pigment called brazilein [20].

3.4. Effect of Temperature on brazilin content

Based on Table 4 and Table 5, temperature affects brazilin content during storage. In control samples without treatment, at room temperature (30°C) there was a decrease in brazilin content by 66% on day 12, while at 5°C and 16°C there was a decrease of 49% and 65%. In samples with treatment, at room temperature storage there was a decrease of 38% and while at 5°C and 16°C there was a decrease of 29% and 30%.

Table 4. Brazilin content of control samples during storage

Time (day)	G30	G5	G16
0	0.81 ± 0.01f	0.81 ± 0.01f	0.81 ± 0.01f
6	0.20 ± 0.00c	0.33 ± 0.02e	0.16 ± 0.24b
12	0.15 ± 0.21a	0.32 ± 0.00d	0.16 ± 0.04b

*Description: Average ± Standard Deviation

In general, the cold temperature of 5°C is the best treatment in maintaining brazilin content during storage for control samples and samples with a combination treatment of blanching and cabinet drying, but in samples with treatment it appears that the decrease in brazilin content is lower than the control sample. This is in accordance with the low storage temperature can activate enzymes, to maintain stability and slow down the degradation of pigment compounds. An increase in temperature can cause color fading [21].

Table 5. Brazilin content of treatment samples during Storage

Time (day)	H30	H5	H16
0	0.51 ± 0.03f	0.51 ± 0.03f	0.51 ± 0.03f
6	0.23 ± 0.04c	0.26 ± 0.07d	0.24 ± 0.16e
12	0.13 ± 0.19a	0.22 ± 0.02b	0.21 ± 0.02b

*Description: Average ± Standard Deviation

The brazilin content in the 5°C and 16°C temperature treatments were not significantly different on day 12 of storage, so it can be concluded that commercially the wedang uwuh RTD drink can be stored at cold temperature (5°C) in a chiller and at AC temperature (16°C). The blanching treatment on the sample also affected the stability of brazilin during storage as evidenced that although the brazilin content of the treated sample was lower than the control on day 0, it could maintain the decrease in brazilin content during storage until day 12. This result is in accordance with another purpose of blanching which is to prevent browning [22]. This is because the blanching treatment can inhibit enzyme activity, thus slowing down the oxidation and degradation of pigment compounds. The decrease in the amount of pigment compound content indicates the degradation process into several degradation products during storage at room temperature and sunlight exposure. The effect of temperature and sunlight exposure accelerates the degradation process of pigment compounds during storage [23].

4 Conclusion

The conclusion of this study is that light and temperature affect the color stability and brazilin content of wedang uwuh RTD beverages during storage. The wedang uwuh material with

water blanching and cabinet drying treatment was more stable than the control (without treatment) both in color stability and brazilin content. Storage of wedang uwuh in brown glass bottles was more effective in maintaining red color and brazilin content for 12 days compared to storage in clear glass bottles. Brown glass bottles protect the beverage from light exposure, especially UV light, which can cause photodegradation and oxidation of brazilin pigments. Therefore, to maintain the quality of wedang uwuh, it is recommended to use brown glass bottles or other packaging that can protect the beverage from light exposure. While the best storage temperature is at cold temperature (5°C). suggestions from further research are shelf life and microbiological testing on wedang uwuh RTD so that it is ready to be commercialized in the market.

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