

## **Brazilin Content, Color Stability, and Antioxidant Activity of Sappan Wood (*Caesalpinia Sappan L.*) Traditional Drink by Different Blanching and Drying Methods**

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### **Abstract**

*Caesalpinia sappan L* (CS) heartwood is used as a natural red colorant in several Asian ethnic traditional drinks both as sole ingredient or mixed with other herbs. However, the pigment is unstable and easily degraded during storage which affects consumer acceptance. This research aimed to investigate the effect of different blanching and drying methods on the color stability, brazilin content, and antioxidant activity of CS heartwood drinks. Steam blanching, water blanching, sun drying and cabinet drying were examined in this study. The most common method applied in traditional production, without blanching and sun drying, was used as a control. The experimental design used was complete randomized design with triplicates. Data are presented as mean  $\pm$  standard deviation and analyzed by SPSS statistics version 20.

The results showed that blanching and drying methods affected the color intensity. For a pure CS heartwood herbal drink, the sample prepared with water blanching and cabinet drying (BCD) showed the highest red value, leading to a 48 % improvement compared to the control. This red value is in accordance with the brazilin content. Sample BCD showed the highest brazilin content, which was 376 % higher than that of the control. Similarly, the BCD sample had the highest antioxidant activity and total phenolic content, with IC<sub>50</sub> values of 98.99 ppm and 213.12 mg GAE g<sup>-1</sup>, respectively. This result revealed that brazilin might act as an antioxidant. Similar to the pure CS drink, the highest quality of mixed herbal drink was obtained by BCD sample. The results suggest that the combination of water blanching and cabinet drying (BCD) was the best method to improve the quality of CS heartwood drink both in pure and mixed herbal drinks as a functional drink.

**Keywords:** *Caesalpinia sappan L.*, Brazilin, Antioxidant activity, Color stability, Mixed herbal drink

### **Introduction**

*Caesalpinia sappan* Linn., or CS heartwood, belonging to the Leguminosae family, is a redwood commonly grown in numerous regions of Southeast Asia. CS heartwood is used as a natural food colorant in various traditional cuisines in Asia, such as herbal tea, dessert, alcoholic beverages, and reddish sauce or condiment. Besides its attractive color, CS heartwood contains bioactive compounds, hence it is used in traditional herbal medicine. The medicinal properties of CS were documented in traditional medical systems such as the Indian Ayurveda, Chinese traditional herbal, and Thai herbal systems [1]. In traditional

beverages, CS heartwood is used either pure or mixed with other herbs such as ginger, cinnamon leaves, nutmeg leaves, clove leaves, and clove stem. The example mixture traditional drink containing CS heartwood are *wedang uwuh* (Java, Indonesia), *bir pletok* (Java, Indonesia), *saraba* (Celebes, Indonesia), and *wedang secang* (Java, Indonesia) and *pathimukam* tea (India) [2].

CS heartwood contains brazilin as a precursor to form a red color, namely, brazilein. Once the CS heartwood is brewed to make a drink, the intensity of the red color does not last long. This color instability creates a challenge, particularly for ready-to-drink (RTD) products. The stability of brazilein can be attributed to several factors, such as light, oxidation, pH, and metal ion interactions [3]. Another factor that might influence the stability of the red color of CS heartwood drink is temperature. Several heat treatments, including blanching and drying, have been suggested to improve the color stability and antioxidant activity of several horticultural products, such as apple pomace [4], ginger and turmeric [5], sugarcane juice [6], herbal juice from *Centella asiatica* [7], pumpkin [8] and ginger leaves [9]. Heat treatment, such as blanching, can inactivate enzymes, causing unacceptable discoloration in dried horticultural products. The inhibition of enzyme activity can also stabilize bioactive compounds, but losses of phenolic compounds can occur due to thermal degradation and/or leaching during blanching, affecting the antioxidant activity [10]. In addition, during drying, prolonged exposure to heat can cause degradation and fading of pigments, leading to a loss of color intensity [11]. Oxidation reactions can also occur during drying, which further contribute to color changes. The drying process can also affect the antioxidant activity of herbs due to the loss of water-soluble antioxidants, preservation of lipid-soluble antioxidants, and the Maillard reaction [12].

In general, sappan wood used in beverages without treatment before drying. Some factors that affect the quality of sappan wood drinks containing brazilin include pH, temperature and light which cause color changes during storage [13,14]. Some research that has been done related to sappan wood as a traditional drink includes the making of sappan wood jelly and jelly drink [15,16], glutinous rice sappan wood drink [17], syrup and instant sappan wood drink [18]. The research conducted is the formulation of several traditional drinks using sappan wood, but no one has examined the treatment of raw materials that are thought to have an effect on the color stability of the drink. The effect of blanching and drying methods on the color stability, brazilin content, and antioxidant activity of brazilin has not been reported. Therefore, this study aimed to investigate the effects of different types of blanching, including steam and water blanching, as well as different types of drying, including sun and cabinet drying, on the stability and antioxidant activity of brazilin in both pure and mixed herbal drinks. The methods of blanching and drying used in this study are selected based on their simplicity and feasibility for application in small-scale industries as the main producers of herbal drinks.

## Materials and methods

### Materials

Fresh CS heartwood (*Caesalpinia sappan* L.) was obtained from Kemuning Forest Gunung Kidul, Yogyakarta. Several ingredients, including fresh ginger, cinnamon leaves, nutmeg leaves and clove leaves, were used to make mixed herbal drinks. These ingredients were obtained from farmers in Samigaluh, Kulon Progo, Yogyakarta. The brazilin standard was purchased from Sigma Aldrich USA, and analytical chemicals were obtained from Sigma Aldrich and Merck.

### Sample preparation

The fresh CS heartwood was chopped into small pieces of approximately 300×100×100 mm<sup>3</sup>. Fresh ginger is sliced in the direction of the rhizome fiber measuring approximately 300×100×50 mm<sup>3</sup>, while fresh leaves are not reduced in size. The sample was divided into single sample (pure CS Heartwood) and

mixed sample (mixed herbal drink). This mixed sample is created from a specific blend of CS Heartwood, ginger, cinnamon leaves, nutmeg leaves, clove leaves, and clove stalks in ratios of 9:5:1:1:1:1, respectively. Initially, the CS Heartwood is cut into blocks and treated according to the procedures outlined in **Table 1**. Each ingredient in the mixed sample undergoes similar treatment as specified in **Table 1**. After treatment, all samples, both single and mixed, are ground into powder form.

The chopped herb was then treated by different blanching and drying methods, as presented in **Table 1**. The sample prepared with sun drying and without blanching was used as a control (C).

**Table 1** Experimental design and selection conditions for sample preparation.

Type of processing	Type of drying	Drying condition		Processing condition	
		Temperature (°C)	Time (h)	Temperature (°C)	Time (min)
Non blanching	Sun drying (C)	40	48	27	1
	Cabinet drying (NBCD)	55	12	27	1
Water Blanching	Sun drying (BSD)	40	48	85	1
	Cabinet drying (BCD)	55	12	85	1
Steam Blanching	Sun drying (SSD)	40	48	100	1
	Cabinet drying (SCD)	55	12	100	1

#### Color analysis

Fifteen milligram samples were brewed in 150 mL of water at 85 °C for 15 min. The sample was then centrifuged at 2,330 g for 15 min. The liquid was transferred into a Petri dish and subjected to a chromameter (Minolta CR-400, Osaka Japan). The results are displayed as numbers via L\* (brightness ranges from 0 - 100), values a\* (from green to red) and b\* (from blue to yellow). The lightness notation is expressed as a brightness parameter with values between 0 - 100 starting from dark to light. Redness notation is expressed as green and red color parameters, values from  $(-80) \pm (+100)$  from green to red and yellowness notation is expressed as color from blue to yellow with a value range from  $(-70) \pm (+70)$ .

#### Determination of brazilin content

The brazilin analysis was carried out using high-performance liquid chromatography (Waters, Massachusetts, USA) comprising a quaternary pump, an on-line degasser, an autosampler and a photodiode array detector. The detector wavelength was set at 286 nm. Separation was achieved using a Reliant C18 analytical column (150, 4.6 and 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) at a flow rate of 1.0 mL min<sup>-1</sup> and run at 40 °C. 10 g for CS heartwoods and 5 g for mixed herbal drink dissolved in 50 and 25 mL MeOH, respectively, followed by sonication extraction at 30 °C for 30 min. The extraction was repeated 3 times. The extraction solvent was evaporated using a rotary evaporator. Subsequently, 10 milligrams of dry extracts were dissolved in 1,000 µL MeOH. The solutions were then diluted as much as 10 and 5 times for pure and mixed herbal drink samples, respectively. The diluted solutions were filtered with 0.22 µm PTFE, and a 5 µL sample was then injected into the high performance liquid chromatography (HPLC).

### **The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay**

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was conducted following a previous study by Baliyan *et al.* [19]. 2 mL of 0.05 mg mL<sup>-1</sup> ethanolic DPPH solution was mixed with 2 mL of sample, followed by 30 min incubation in a dark place at room temperature. The absorbance values were measured at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV 1280, Kyoto, Japan).

### **The 2,20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Radical Scavenging Method**

The 2,20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)(ABTS) radical scavenging analysis refers to the method carried out by Kulczyński *et al.* [20]. The analysis was carried out by mixing 30 µL of extract and 3 mL of ABTS reagent. The mixture was then homogenized for 6 min. The measurements were carried out at a wavelength of 734 nm. A mixture of extraction reagent and ABTS solution was used as a control. The results obtained were expressed as mg Trolox equivalent (Tx) per 100 g dry mass.

### **Determination of total phenolic content (TPC)**

The total phenolic compound was determined according to Velioglu *et al.* [21] with minor modification. Gallic acid solutions with concentrations of 0.00, 0.02, 0.04, 0.06, 0.08, and 0.10 mg mL<sup>-1</sup> were used to generate standard curves. 5 mL of 2 % Na<sub>2</sub>CO<sub>3</sub> was added to 1 mL of sample or standard, mixed, and incubated for 10 min. Subsequently, 0.5 mL of 50 % Folin-Ciocalteu solution was added, mixed, and incubated for 30 min. The absorbance of the sample was determined at a wavelength of 760 nm using a UV-Vis spectrophotometer UV-VIS Spectrophotometer Shimadzu UV-1280, Japan, Shimadzu Corporation.

### **Determination of total flavonoid content (TFC)**

The total flavonoid content (TFC) was determined according to a previous study [22]. A 0.5 mL aliquot of sample was added to 1.5 mL of aluminum chloride (5 %) along with 1 mL of sodium acetate buffer (pH 5.5). The solutions were mixed and incubated at 25 °C in the dark for 20 min. The absorbance was measured at 400 nm.

### **pH measurement**

pH measurements on pure CS heartwood and mixed herbal drink samples were carried out during storage using a pH meter (Ohaus, New Jersey, USA).

### **Statistical analysis**

The experimental design used was complete randomized design with triplicates. Data are presented as mean ± standard deviation and analyzed by SPSS statistics version 20. The antioxidant activity values (DPPH, ABTS), L\*, a\*, b\* values and brazilin content were subjected to an one-way analysis of variance (ANOVA) followed by a post-hoc Duncan Multiple Range Test (DMRT). The significance level was set at 0.05 ( $\alpha = 0.05$ ). Meanwhile, testing of pH and color changes in samples during storage was carried out at room temperature (27 °C) for 15 days and tested every 3 days. The data obtained were analyzed and graphed using Microsoft Excel.

## Results and discussion

### Effect of blanching and drying on the color stability of CS heartwood both in pure and mixed herbal drinks

The shift towards a healthy lifestyle post-COVID pandemic has resulted in an increase of consumers' preference for functional drink including sappan wood drink. The commercial sappan wood drinks are generally in the form of simplicia and powder [23]. In order to increase product variety of sappan wood and fulfill consumer's demand for more practical products, sappan wood can be developed into a RTD product. One of the challenges in making RTD is the color change of the product during storage from red to brown which affects consumer acceptance. Several attempts have been made to improve the stability of the reddish color of herbal drinks containing CS heartwood, such as methylation, encapsulation, and changes in pH [3,24,25]. However, this approach is not practically and economically favorable. Blanching has been used for a long time to increase color intensity in several fruits and vegetables, but few studies have investigated the effect of blanching on herbs. To the best of our knowledge, there is no report on the effect of blanching on CS heartwood. Brazilin is sensitive to oxygen and light; therefore, the method of drying might affect color intensity. In practice, CS heartwood is usually used in a mixed herbal drink as a colorant; hence, in this study, both pure and mixed herbal drinks were investigated. One of the most popular mixed herbal drinks containing CS heartwood is *wedang uwuh*. *Wedang uwuh* contains CS heartwood, ginger, cinnamon leaves, nutmeg leaves, and clove leaves, in which cloves, cloves leaves, and ginger have a yellow color, which might affect the overall color of the mixed herbal drink. In this study, different approaches using steam and water blanching as well as sun and cabinet drying are proposed, as these methods are feasible for application in small-scale production.

The color intensity was determined using a chromameter, and the results are presented in **Table 2**. The results show that both the blanching and drying methods affect the color intensity. The highest red color intensity shown by the  $a^*$  value was obtained from the water blanching and cabinet drying (BCD) sample, which increased by 48 % compared to that of the control in the pure CS heartwood drink. Samples with cabinet drying increased the red color intensity by 26 - 54 % compared with those using sun drying. Meanwhile, samples prepared with blanching methods only improved by 18.5 - 24.7 % compared with no blanching samples. This indicates that the effect of the drying method was more pronounced than the effect of the blanching methods. The red value of samples prepared with cabinet drying was 3.65 - 4.35, while the value for sun drying varied from 2.82 - 3.65. This result reveals that cabinet drying showed a higher color intensity than sun drying. This could be explained by lower exposure to oxygen and light during drying using a cabinet dryer than during sun drying. The cabinet dryer needed less time to achieve the same moisture compared to sun drying; hence, it gave lower exposure to oxygen. As seen in **Table 2**, the no blanching samples had a lower red color intensity (2.93 - 3.65) than the blanching samples (2.82 - 4.35). The result shows that blanching increased the intensity of red color compared to no blanching samples. This might be explained by the inactivation of the polyphenoloxidase (PPO) enzyme [26]. This enzyme has been reported to degrade natural pigments. The water blanching method resulted in a higher color intensity than steaming blanching, which could be attributed to the lower temperature of water blanching since PPO is reported to be inactivated by mild heating [27]. The highest color intensity was obtained from the sample with the combination of water blanching and cabinet drying treatment in both pure and mixed herbal drinks containing CS heartwood. So far there has been no color research on sappan wood and mixed samples treated with blanching, but there are studies that show sappan wood without treatment has an  $a^*$  value of  $0.36 \pm 0.05$  -  $0.46 \pm 0.09$  [28] while mixed herbal drink samples (without heat treatment) called *wedang uwuh* in Indonesia in some studies show a sharp decrease in color during storage [13,18].

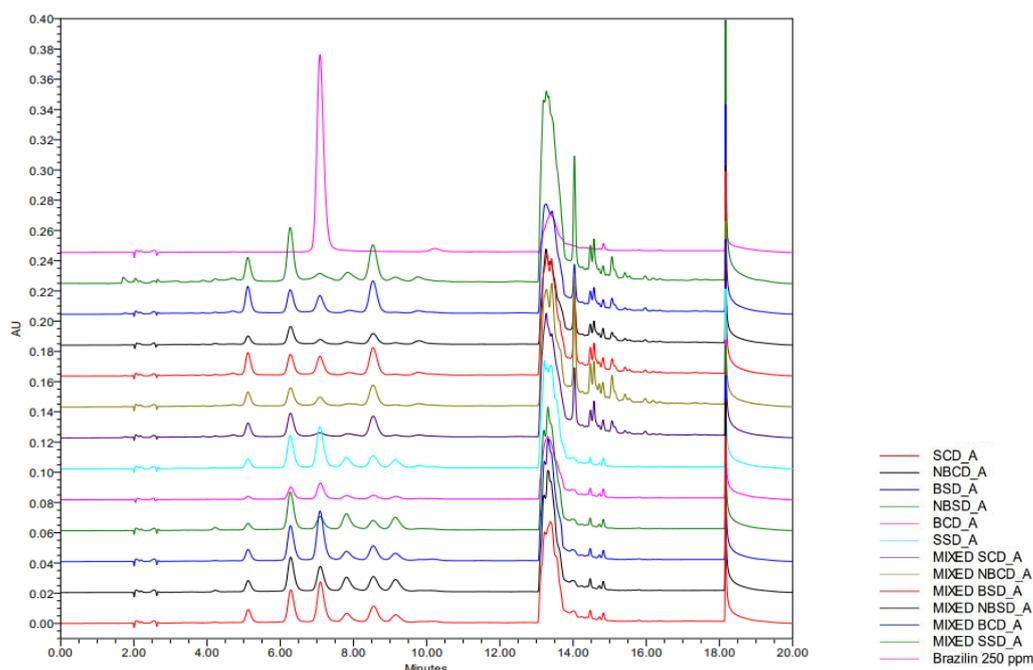
**Table 2** Color intensity of pure CS heartwood drink and mixed herbal drink.

Processing condition	L*		a*		b*	
	Pure	Mixed	Pure	Mixed	Pure	Mixed
C	33.10 ± 0.05 <sup>f</sup>	23.71 ± 0.02 <sup>c</sup>	2.93 ± 0.23 <sup>b</sup>	5.29 ± 0.10 <sup>b</sup>	3.62 ± 0.45 <sup>b</sup>	4.31 ± 0.08 <sup>c</sup>
NBCD	32.46 ± 0.67 <sup>c</sup>	23.67 ± 0.08 <sup>b</sup>	3.67 ± 0.40 <sup>c</sup>	4.4 ± 0.08 <sup>a</sup>	4.55 ± 0.67 <sup>d</sup>	3.35 ± 0.14 <sup>a</sup>
BSD	31.74 ± 1.04 <sup>b</sup>	23.25 ± 0.07 <sup>a</sup>	2.82 ± 0.78 <sup>a</sup>	7.49 ± 0.06 <sup>c</sup>	3.72 ± 0.56 <sup>c</sup>	4.67 ± 0.11 <sup>c</sup>
BCD	32.15 ± 0.98 <sup>c</sup>	23.71 ± 0.04 <sup>c</sup>	4.35 ± 0.35 <sup>d</sup>	8.79 ± 0.15 <sup>d</sup>	5.30 ± 0.23 <sup>e</sup>	6.04 ± 0.18 <sup>f</sup>
SSD	32.22 ± 0.23 <sup>d</sup>	23.66 ± 0.12 <sup>b</sup>	3.65 ± 0.20 <sup>c</sup>	7.53 ± 0.12 <sup>c</sup>	3.13 ± 0.40 <sup>a</sup>	4.55 ± 0.25 <sup>d</sup>
SCD	31.70 ± 0.45 <sup>a</sup>	23.74 ± 0.16 <sup>d</sup>	3.65 ± 0.24 <sup>c</sup>	5.16 ± 0.42 <sup>b</sup>	3.13 ± 0.98 <sup>a</sup>	4.15 ± 0.09 <sup>b</sup>

Values are presented as the mean value (n = 3). Different letters in the same column indicate significant differences ( $p < 0.05$ ).

### Effect of blanching and drying treatment on brazilin

Brazilin is a natural heterocyclic crystalline substance that is found in different plant species such as *Caesalpinia sappan* L., *Haematoxylum brasiletto*, *Caesalpinia crista*, and *Haematoxylum campechianum*. There is a limited study that analyses brazilin in real samples. In the current work, the brazilin content was determined by HPLC. Linearity between concentration and peak area was tested using the regression line as provided by the least square analyses. The correlation coefficient ( $r^2$ ) of the standard curve was  $> 0.99$ , which was considered linear. The limit of detection and the limit of quantification obtained in this study were 0.006 and 0.02  $\mu\text{g mL}^{-1}$ , respectively. The chromatograms of the brazilin standard and samples are presented in **Figure 1**.



**Figure 1** Chromatogram of brazilin both in standard and samples with different methods of blanching and drying.

The brazilin content of the samples can be seen in **Table 3**. The result shows that the highest brazilin content in pure and mixed CS heartwood was obtained from samples treated with water blanching and cabinet drying (BCD). This result is in accordance with the color intensity results. This indicates that brazilin plays a role in the color intensity of CS heartwood in both pure and mixed drinks. The highest brazilin contents obtained in the current study are 1.3152 and 0.1889 % in pure and mixed CS heartwood drinks, respectively. The similar results of pure and mixed herbal drinks reveal that CS heartwood plays an important role in the color of mixed herbal drinks. This value is lower than a previous study by Masaenah *et al.* [29], which reported that the brazilin content of *Caesalpinia sappan Linn* was 2.13 %, but equal with research by Arsiningtyas [30] that brazilin content from methanol extractan was 1.30. The different values could be attributed to the different sources of raw samples and solvents used for extraction. In a previous study, brazilin was extracted 2 times using ethanol and methanol, whereas in this study, brazilin was extracted using water

Blanching and cabinet drying increased the brazilin content, in which samples treated with blanching and cabinet drying had 3x and 1.5x higher brazilin than that of the control, respectively. One reason for the higher brazilin content in samples treated with water blanching might be the decrease in PPO enzyme activity. Samples treated with water blanching showed higher brazilin content than those treated with water blanching. This could be explained by the lower temperature of water blanching since the PPO enzyme can be inactivated at mild temperatures. The higher brazilin content in the sample treated with cabinet drying might be due to the lower exposure of light and oxygen to the samples.

**Table 3** Brazilin content in pure CS heartwood drink and mixed herbal drink with different blanching and drying methods.

Sample	Brazilin content (%)					
	C	NBCD	BSD	BCD	SSD	SCD
Pure sample	0.28 ± 0.01 <sup>a</sup>	0.66 ± 0.01 <sup>b</sup>	1.11 ± 0.01 <sup>d</sup>	1.31 ± 0.01 <sup>e</sup>	1.01 ± 0.01 <sup>e</sup>	1.02 ± 0.06 <sup>d</sup>
Mixed sample	0.08 ± 0.01 <sup>c</sup>	0.12 ± 0.01 <sup>d</sup>	0.18 ± 0.01 <sup>e</sup>	0.19 ± 0.01 <sup>f</sup>	0.03 ± 0.01 <sup>a</sup>	0.04 ± 0.01 <sup>b</sup>

Values are presented as the mean value (n = 3). Different letters in the same row indicate significant differences ( $p < 0.05$ ).

#### Effect of blanching and drying on the antioxidant activity of pure CS heartwood and mixed herbal drink

In addition to affecting color, blanching and drying methods might influence antioxidant activity since antioxidant compounds are commonly sensitive to temperature. The antioxidant activities were measured using DPPH and ABTS methods, and the results are represented as IC<sub>50</sub> values. **Table 4** shows that the results of DPPH and ABTS were in line. The result shows that both the blanching and drying methods affected the antioxidant activity. Blanching increased the antioxidant activity in the range of 83.33 - 95.76 % and 25.42 - 51.02 % for sun drying and cabinet drying, respectively. Meanwhile, the antioxidant activity of cabinet drying is 36.02 % higher than that of sun drying. Samples treated with water blanching and cabinet drying had higher antioxidant activity, with IC<sub>50</sub> values of 98.99 ppm (DPPH) or 4.56 ppm (ABTS). This value is lower than that reported by Arsiningtyas [30] when tested using DPPH methods but higher than the result from research by Setiawan *et al.* [31]. These differences in values could be due to differences in sample origin, treatment processes, and extraction methods. However, based on references, CS heartwood

extract has strong to very strong antioxidant activity. The result of antioxidant activity is in accordance with the brazilin content of the sample. It has been reported that brazilin has antioxidant activity [1,32,33]. In addition, phenolic compounds and flavonoids are responsible for antioxidant activity [34]. Therefore, blanching and drying methods might affect phenolic compounds and flavonoids.

**Table 4** Free radical scavenging activity of pure CS heartwood drink and mixed herbal drink with different blanching and drying methods.

Sample	IC <sub>50</sub> (ppm)					
	C	NBCD	BSD	BCD	SSD	SCD
Pure sample						
DPPH	231.89 ± 5.73 <sup>c</sup>	148.36 ± 2.48 <sup>d</sup>	126.26 ± 1.00 <sup>c</sup>	98.99 ± 1.099 <sup>a</sup>	118.21 ± 1.41 <sup>b</sup>	118.09 ± 0.42 <sup>b</sup>
ABTS	7.97 ± 0.23 <sup>c</sup>	7.53 ± 0.05 <sup>c</sup>	7.14 ± 0.25 <sup>b</sup>	4.56 ± 0.15 <sup>a</sup>	6.19 ± 0.28 <sup>b</sup>	6.21 ± 0.22 <sup>b</sup>
Mixed sample						
DPPH	235.05 ± 1.56 <sup>e</sup>	269.42 ± 0.99 <sup>f</sup>	131.62 ± 0.75 <sup>b</sup>	126.51 ± 0.83 <sup>a</sup>	161.34 ± 0.60 <sup>d</sup>	141.16 ± 1.57 <sup>c</sup>
ABTS	19.53 ± 31.56 <sup>d</sup>	16.69 ± 0.35 <sup>c</sup>	14.553 ± 1.25 <sup>b</sup>	10.99 ± 0.62 <sup>a</sup>	14.42 ± 0.41 <sup>b</sup>	11.01 ± 1.14 <sup>a</sup>

Values are presented as the mean value (n = 3). Different letters in the same row indicate significant differences ( $p < 0.05$ ).

In this study, the effect of blanching and drying can increase the antioxidant activity in CS heartwood and mixed herbal drink. This was similar in the study with *Diospyros kaki* L. [35], but in *Dawrach* (*Raphanus Raphanistrum* L.) sample, blanching treatment decreased antioxidant activity [36]. While in the *Moringa oleifera* leaf sample, blanching treatment or without blanching did not show different results on antioxidant activity [37].

#### Total phenolic and flavonoid contents of pure and mixed herbal drinks containing brazilin

Phenolic compounds commonly found in different parts of plants have been reported to possess antioxidant properties [38]. CS heartwood contains various phenolic compounds, such as sappanchalcone, caesalpinaphenol G, quercetin [39], brazilin, xanthone, flavones and coumarin [34,40], which are able to contribute to hydrogen atom donor and electron donor mechanisms in scavenging free radicals [41]. Heat treatment is reported to affect phenolic compounds; hence, blanching and drying might influence the total phenolic compound (TPC) and total flavonoid (TF) contents. **Table 5** shows that the effect of drying on a pure sample has an influence on TPC. The cabinet drying treatment gave a higher TPC value than sun drying. One reason could be due to the longer exposure of oxygen to sun drying, which increases PPO activity, thus lowering the TPC. Meanwhile, the effect of blanching depends on the method used, as shown in samples treated by sun drying. Samples treated with water blanching had lower TPC, whereas samples treated with steam blanching had higher TPC. The lower TPC content in water blanching could be due to the solubility of phenolic compounds. Meanwhile, the higher TPC content in steam blanching could be attributed to the lower activity of PPO to degrade phenolic compounds. Blanching can inactivate the polyphenol oxidase enzyme involved in flavonoid degradation. This enzyme is responsible for the oxidation of flavonoids, which can reduce the flavonoid content in foods. By inactivating the polyphenol oxidase enzyme, blanching can prevent flavonoid degradation, thereby increasing the flavonoid content in food. In mixed samples, the amount of TPC increased due to the presence of other ingredients, such as ginger,

cinnamon leaves, nutmeg leaves and clove leaves, which contributed phenol compounds. These ingredients have been reported to contain phenolic compounds such as gingerols and shogaols in ginger [42], eugenol in clove leaves [43], sabinene, eugenol, myristicin, caryophyllene,  $\beta$ -myrcene, and  $\alpha$ -pinene in nutmeg leaves [44], and trans-cinnamaldehyde in cinnamon leaves [45]. The total flavonoid content was in line with the total phenolic compound and brazilin contents. This indicates that brazilin, as a neoflavonoid compound, is the major flavonoid compound in CS heartwood [3].

**Table 5** Total phenolic content and total flavonoid content of pure CS heartwood drink and mixed herbal drink with different blanching and drying methods.

Sample	Processing condition					
	C	NBCD	BSD	BCD	SSD	SCD
Pure sample						
TPC	151.78 $\pm$ 0.57 <sup>b</sup>	208.70 $\pm$ 0.34 <sup>d</sup>	113.83 $\pm$ 0.54 <sup>a</sup>	213.12 $\pm$ 0.87 <sup>d</sup>	186.02 $\pm$ 0.54 <sup>c</sup>	218.19 $\pm$ 0.87 <sup>d</sup>
TFC	12.43 $\pm$ 1.96 <sup>a</sup>	15.69 $\pm$ 3.93 <sup>a</sup>	12.00 $\pm$ 4.00 <sup>a</sup>	16.09 $\pm$ 7.18 <sup>a</sup>	8.81 $\pm$ 5.76 <sup>a</sup>	14.10 $\pm$ 4.44 <sup>a</sup>
Mixed sample						
TPC	563.85 $\pm$ 5.74 <sup>b</sup>	576.09 $\pm$ 5.77 <sup>b</sup>	443.14 $\pm$ 5.64 <sup>a</sup>	738.52 $\pm$ 0.01 <sup>c</sup>	727.93 $\pm$ 5.71 <sup>d</sup>	572.39 $\pm$ 5.30 <sup>bc</sup>
TFC	4.68 $\pm$ 2.03 <sup>a</sup>	10.46 $\pm$ 2.26 <sup>a</sup>	20.00 $\pm$ 5.77 <sup>b</sup>	23.90 $\pm$ 2.18 <sup>b</sup>	6.29 $\pm$ 2.18 <sup>a</sup>	6.17 $\pm$ 2.14 <sup>a</sup>

Values are presented as the mean value (n = 3). Different letters in the same row indicate significant differences ( $p < 0.05$ ).

Blanching, as an effective pretreatment method for controlling enzymatic browning, is widely used before further processing (including freezing, canning, juicing, and drying) due to its low cost and simple operation. Steam and hot water blanching are still considered efficient methods to inactivate endogenous browning-related enzymes. For blanching pretreatment, it is essential to balance the inactivation of endogenous enzymes in the sample that cause undesirable quality attributes and the retention of nutrients, bioactive compounds, and functional properties [46].

#### **Effect of blanching and drying on pH and color during the storage of pure CS heartwood and mixed herbal drink**

The color instability of CS heartwood drink during storage is an important issue, as color is influenced by pH. CS heartwood appears red at neutral pH (6 - 7), yellow at acidic pH (2 - 5), and purplish red at base pH (above pH 8) [47]. During storage, microbial activity might produce acid and thus reduce the pH. Blanching and drying could reduce the microbial load; hence, they might influence the pH and color of CS heartwood during storage. The effects of blanching and drying on the pH of pure and mixed herbal drinks are presented in **Figures 2** and **3**, respectively. Meanwhile, the color change of pure and mixed herbal drinks are presented in **Figures 4** and **5**, respectively. The results show that blanching treatment of pure and mixed herbal drink samples can maintain the pH during 15 days of storage. Meanwhile, the pH of the control decreased starting from Day 3 and continued to decrease over 15 days. The decrease of the pH is due to microbial activity. Blanching might reduce the microbial load on the surface of the material, thus maintaining the pH and prolonging the shelf life of the product. Blanching treatment can be an efficient and low-cost alternative approach to plant preservation.

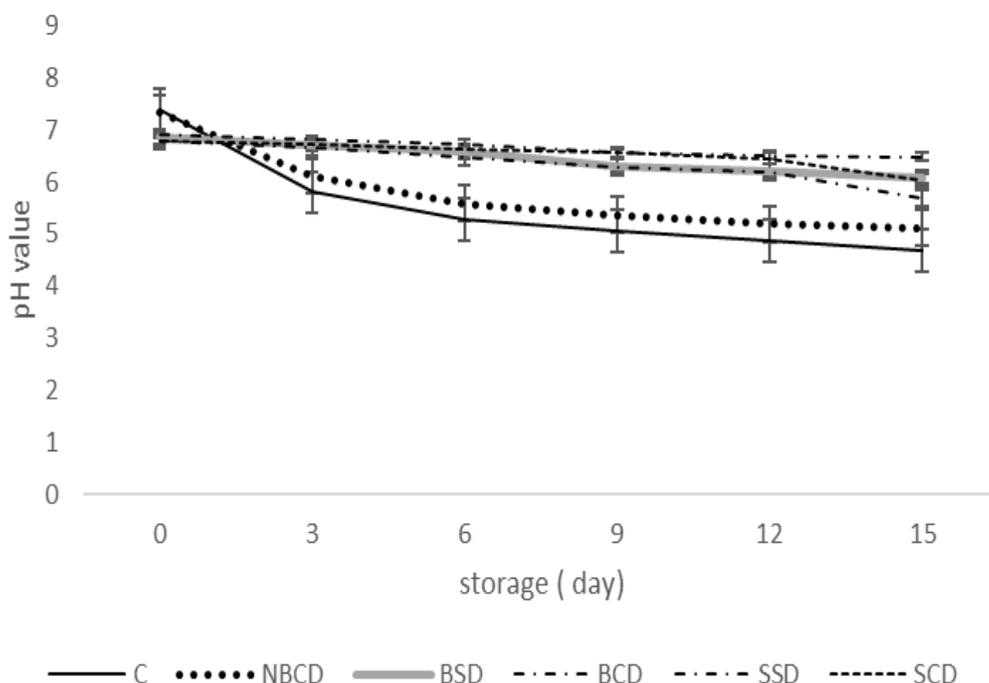


Figure 2 pH of pure CS heartwood drink during storage with different blanching and drying methods.

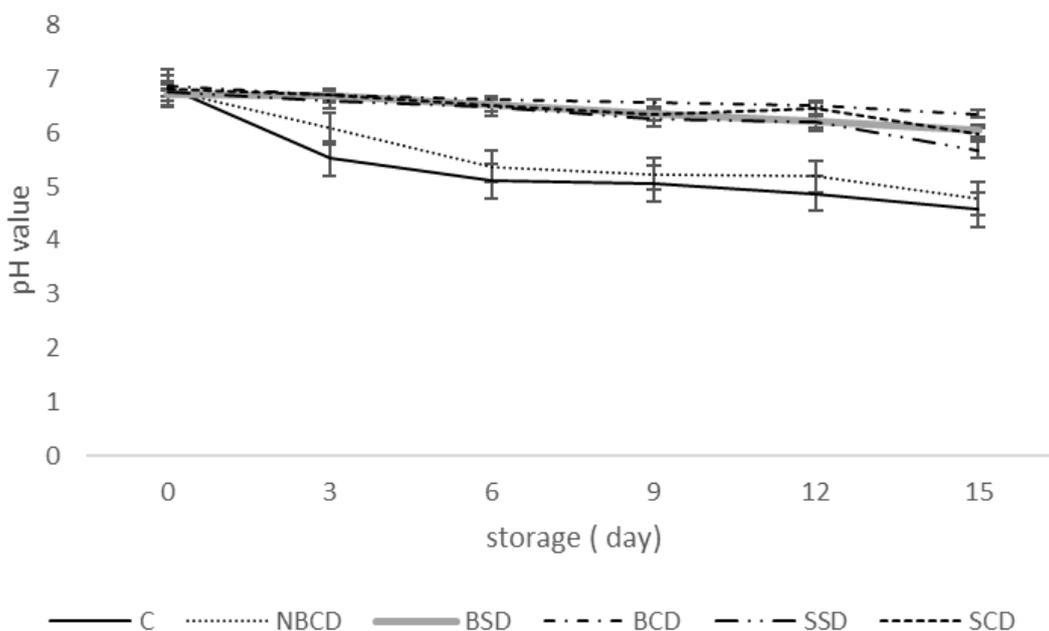
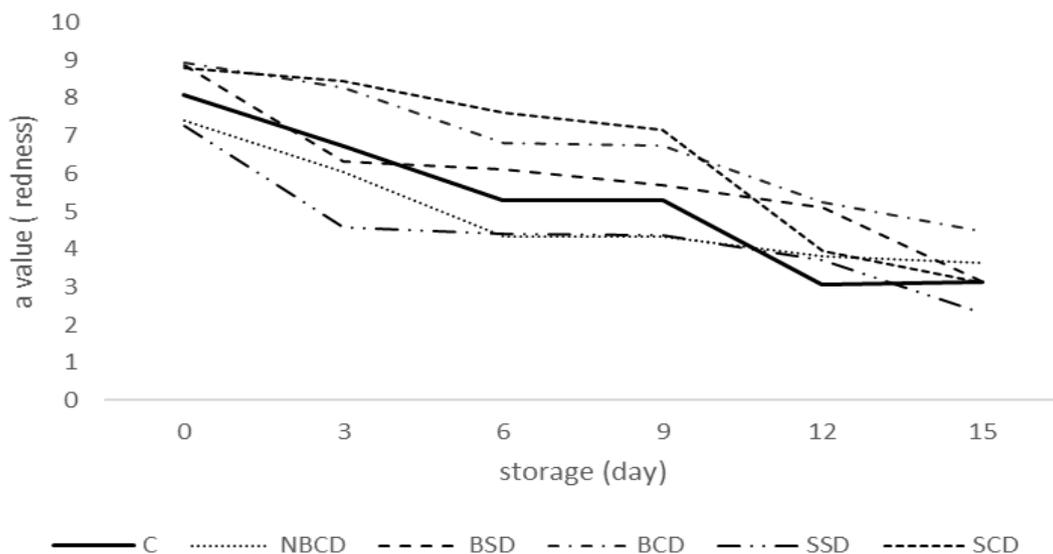
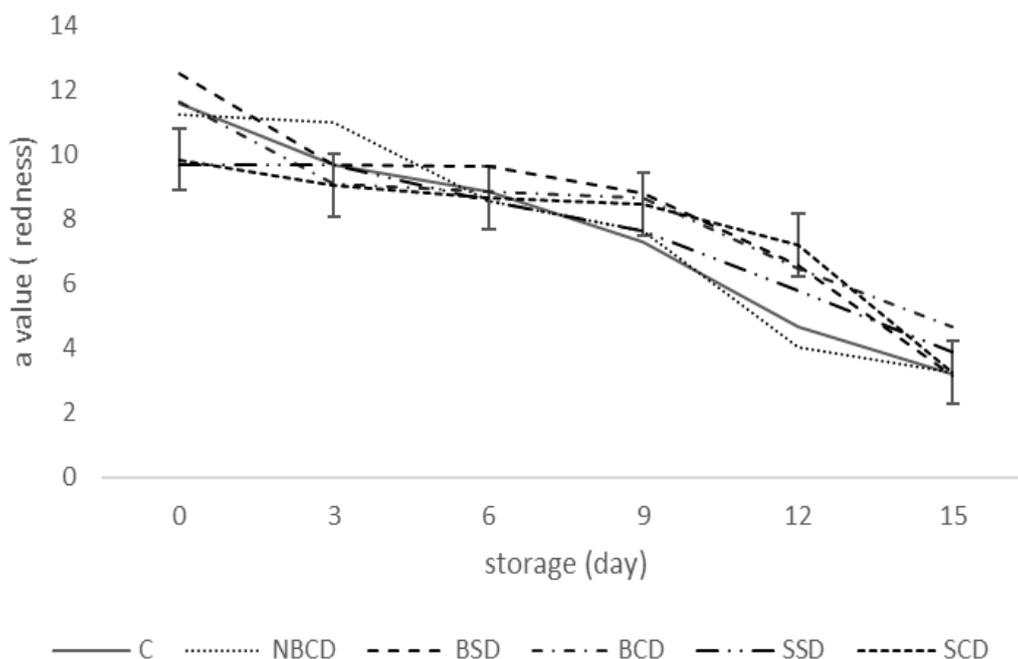


Figure 3 pH of mixed herbal drink during storage with different blanching and drying methods.

The effect of blanching and drying on the color of the mixed CS heartwood drink is presented in Figure 5. The results show that the color stability of all samples decreases during storage. A higher decrease is observed on the samples without blanching. In addition, the highest color stability was obtained with treatment using cabinet drying.



**Figure 4** Color change during storage in the pure CS heartwood drink with different blanching and drying method.



**Figure 5** Color change during storage in the mixed herbal drink with different blanching and drying methods.

**Conclusions**

Blanching and drying methods influenced the brazilin content, color intensity, antioxidant activity and color stability of Sappan wood RTD during storage. The best method to improve both for sensory and functional properties was water blanching and cabinet drying (BCD). The effect of blanching and drying method was similar both in pure and in a mixture herbal drink. This result suggests that water blanching

and cabinet drying (BCD) were low-cost method to improve the quality of CS heartwood drinks in both pure and mixed herbal drinks. The mechanism underlying the stability improvement of brazilin content by water blanching and cabinet drying is of interest for further study.

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