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EFFECT OF THERMOSONICATION ON FERRIC REDUCING ANTIOXIDANT POWER AND THE COLOR OF BERAS KENCUR DRINK

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ABSTRACT

Beras kencur drink is an Indonesia's traditional beverage containing several antioxidant compounds that are good for human health. Thermosonication technology was chosen to process the beras kencur drink. This research aimed to evaluate antioxidant activity and the color of beras kencur drink thermosonication with compared to the control untreated and pasteurization treatment. The thermosonication treatment used a probe at a frequency of 22 kHz, while conventional pasteurization conditions were 100^{o}C for 2 min. This study employed a Completely randomised design (CRD) with two factorials; temperatures of thermosonication (30, 45 and 60 ^{o}C) and times of thermosonication (5, 10, 15 min). The data were analyzed by ANOVA followed by DMRT with p-value greater than 0.05. The results showed that the effects of thermosonication on antioxidant activities and the color of beras kencur drink were more retention than the pasteurization treatment. The value antioxidant activity of beras kencur drink treated with thermosonication ranged from 1164.80 – 1323.86 mgAA/L, and the color values ranged from 58.16 – 62.58 for lightness (L*), 0.42 – 1.30 for redness (a*), 20.70 – 22.53 for yellowness (b*) and 0.50 – 5.29 for total color difference.

Keywords: Antioxidant, Beras kencur drink, Color, Thermosonication

INTRODUCTION

Traditional herbal drinks in each region are of different types, one of the herbs that are widely known and favored by the Indonesian people is beras kencur drink. Beras kencur drinks are made from a mixture of spices such as kencur, ginger, and several other functional ingredients. Beras kencur drink contains several antioxidant compounds such as phenols and flavonoids that are good for human health (Suwarno *et al.,* 2022). Secondary metabolites in beras kencur drink can provide various pharmacological effects. According to Latifah and Andrie (2014) beras kencur drink has antidiabetic activity in streptozotocin-induced rats which is characterized by decreased blood glucose levels, weight control and was able to reduce damage to the islets of Langerhans.

Thermal pasteurization methods is a physical preservation technique used to longer shelf-life of food. Unfortunately, higher temperatures and time treatment conduct loss of nutrients, bioactive compounds and decrease in fresh taste (Dolas *et al.,* 2019). Akbar & Murtini (2018), reported that high temperature and heating time have significant changes in the color profile of sugarcane juice drinks. Treatment using heating such as blanching and pasteurization improve the microbiological qualities and antioxidant compound of beras kencur drink, but it can damage the texture and this condition is undesirable (Kiptiyah *et al.,* 2017).

Currently, new processing technologies using non-thermal have been innovatively developed in the food industry, mainly thermosonication technology. Thermosonication combines the ultrasound treatment with moderate heat (Dolas *et al.,* 2019). Thermosonication treatment can inactivate microbes up to 5-log reduction to achieve Food and Drug Administration (FDA) regulations (Bermúdez-Aguirre & Barbosa-Cánovas, 2012). Thermosonication has minimal impact on the qualities, sensory profile, and nutritive value of food compared the conventional heating (Manzoor *et al.,* 2021). Another non-thermal technology using pulsed electric field (PEF) showed that reduction number of microorganisms resulted 3.406 log CFU/mL and there is no significant differences in sensory properties of milk between combination pre-heating and PEF compared to conventional pasteurization (Esfandiar *et al.,* 2022). Previously, several studies have reported that thermosonication treatment can improve the quality of food products such as carrot juice (Jabbar *et al.,* 2015), pear juice (Saeeduddin *et al.,* 2015), and apple juice (Abid *et al.,* 2014).

According to our knowledge, no information about the effect of thermosonication treatment on Ferric Reducing Antioxidant Power (FRAP) and the color of beras kencur drink has been a scarcity. Hence, the outcome of this experimental was to know the thermosonic treatment effect on antioxidant activity and the color of beras kencur drink. The output of this research is that thermosonication can be used as an alternative to thermal processes that can maintain the quality of the kencur rice drink.

METHODOLOGY

Materials

The main ingredients for making beras kencur drink were kencur (*Kaempferia galanga*), ginger (*Zingiber officinale var. amarum*), rice (*Oryza sativa L*), tamarind (*Tamarindus indica*), kedawung (*Parkia timoriana*) and coconut sugar were purchased from a local market (Puspa Agro market, Sidoarjo city, Indonesia). Materials for testing antioxidant activity were monobasic potassium phosphate (KH_2PO_4), dibasic potassium phosphate (K_2HPO_4), Potassium ferricyanide (III) [$K_3Fe(Cn)_6$], ascorbic acid, oxalic acid were pro analysis grade and obtained from Sigma Aldrich (St. Louis, MO, USA). Trichloroacetic acid, Iron(III) Chloride hexahydrate ($FeCl_3.6H_2O$) was purchased from EMSURE® Merck KGaA (Darmstadt, Germany). Ethanol 96% was purchased from Smart lab (Tanggerang, Indonesia).

Equipment

The equipment for making beras kencur drink were blenders, knives, scales, stoves, muslin cloth and a sonication bath (chamber) with a thermocouple and double blower. The equipment for testing the color were colorimeter X (Smyk Serhii, USA) and antioxidant activity using UV-Vis spectrophotometry (Thermo scientific, USA).

Research Design

This experiment was to determine the effect of thermosonication treatment on ferric reducing antioxidant power (FRAP) and the color of beras kencur drink. This experiment used a Completely Randomised Design (CRD) with two factors such as temperatures of termosonication (30, 45 and 60 ^oC) and times of thermosonication (5, 10, 15 min). All the analyses were performed in three replications.

Research Stages

This research was conducted in three stages.The research stages include:

1. Preparation of Beras Kencur Drink

The preparation of beras kencur drink was treated using the Kiptiyah *et al.* (2017) method, with slight modification. Preparation of beras kencur drink, starting with the rice according to formulation, was soaked in water for 12 hours. The kencur and ginger were cleaned and then weighed according to formulation, while tamarind and coconut sugar were

weighed according to formulations then added to water and boiled. Kedawung seeds broken skin and weigh according to formulation. Furthermore, all ingredients were mixed in a blender for ± 3 min, then filtered to separate the coarse particles and get the filtrate of the kencur rice drink.

2. Termosonication Treatment of Beras Kencur Drink

The thermosonication treatment was used by Jabbar *et al.* (2015) with modification. Thermosonication treatment beras kencur drink was began with sterilizing the sonication chamber. The Sonication chamber had a maximum tank capacity of 5 L fluids. Beras kencur drink sample (1 L) was placed in a sonication chamber. The thermosonication processor of 365 W set at frequency of 22 kHz with temperatures according to treatment (30, 45 and 60 ^{o}C), and then the heating button was turned on. After the temperature was reached, the time sonication was started according to treatment (5, 10 and 15 min). Then beras kencur drinks were kept in sterilized bottles for further analysis.

3. Pasteurization Treatment of Beras Kencur Drink

The pasteurization treatment beras kencur drink was treated using the Kiptiyah *et al.* (2017) method. Briefly, beras kencur drink sample (1 L) was placed in a pot and heated using a stove until the temperature reached 100 ^{o}C. During the heating process the sample was continuously stirred. After the temperature was reached, the time pasteurization was started for 2 min. Then pasteurized beras kencur drink was allowed to stand for a while until the temperature was not too hot then put in a sterilized bottle for further analysis.

Methods

Data of the result were expressed as mean ± standard error deviation (SD). Statistical data processing was using IBM® SPSS® Statistics 25 software. One-way analysis of variance (ANOVA) followed by the Duncan Multiple Range Test (DMRT) with a p-value of 0.05 was used to determine significant differences among variations of treatment. All test were measured in triplicate.

Analysis Procedure

1. Determination of Ferric Reducing Antioxidant Power (FRAP) Beras Kencur Drink

Antioxidant activity was analyzed by Raharjo and Haryoto (2019) with the FRAP method. Briefly, 0.2 M phosphate buffer (pH 6.6) and 6% $K_3Fe(CN)_6$ 1 mL each were added to a known concentration of the sample (1mL), and then incubated for 20 min at 50 °C. After incubated, TCA (1mL) was added and centrifuged (3000 rpm for 10 min). After that, the top layer was pipetted (1 mL) and put in another test tube. The distilled water (1mL) and 0.1% $FeCl_3$ (0,5 mL) were added and then the solution was left for 10 min. The absorbance sample was quantified at λ 720 nm and calculated with an ascorbic acid standard curve. The results were expressed as mg equivalent of ascorbic acid / L.

2. Determination of color Beras Kencur Drink

The color of beras kencur drink samples was measured using a colorimeter based hunter system on three color coordinates, including L* for lightness/darkness, a* for redness/greenness and b* for yellowness/blueness. All the measurements were carried out in triplicate at room temperature. Additionally, color values of L*, a*, and b* were converted in total color difference (ΔE) by the following formula:

$$\text{Total color Difference } (\Delta E) = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

Information:

ΔE = Total color difference
ΔL = difference L* values according to the control
Δa = difference a* values according to the control
Δb = difference b* values according to the control

RESULTS AND DISCUSSION

1. Antioxidant Activity

In this study, antioxidant activity was measured using the Ferric Reduction Antioxidant Power (FRAP) method. Ascorbic acid was used as a standard curve because it's one of the secondary antioxidants that can capture free radicals and prevent chain reactions. The principle of the FRAP method was antioxidant compounds to donate electrons, so that reduced Fe^{3+} ions to Fe^{2+} (Raharjo & Haryoto, 2019). The regression results of calibration curve ascorbic acid absorbance was obtained y = 2.85x - 0.07 with the value R^2 = 0.99 where the concentration (x) and absorbance (y) (Fig 1).

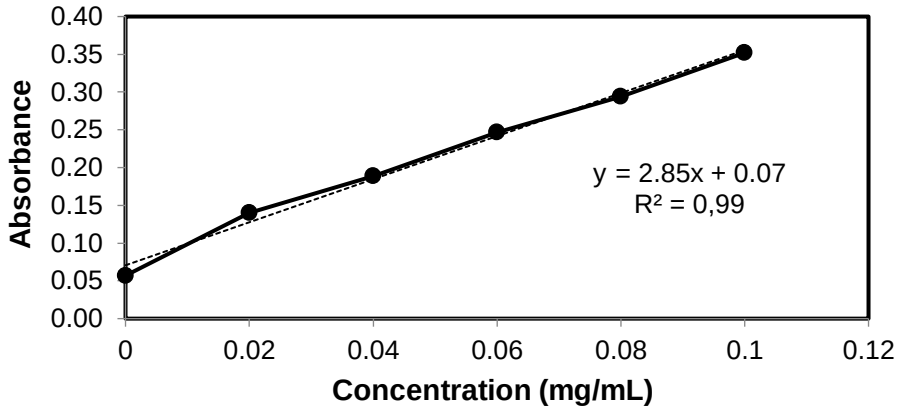

Figure 1. Standard curve of ascorbic acid

The FRAP value was measured by entering the absorbance value of the sample into the regression equation, and expressed in mg AAE/L. The resulting FRAP value of beras kencur drink was presented in Table 1.

Table 1. The measurement result of absorbance and antioxidant activity value of beras kencur drink

Sample	Absorbance			Antioxidant Activity (mgAAE/L)
	Replication 1	Replication 2	Replication 3	
Untreated	0.44	0.44	0.44	1300.47 ± 4.05^{c}
Pasteurized	0.33	0.33	0.33	902.81 ± 3.51^{j}
TS30-5	0.45	0.45	0.45	1323.86 ± 4.05^{a}
TS30-10	0.45	0.44	0.44	1309.82 ± 3.51^{b}
TS30-15	0.44	0.44	0.44	1302.81 ± 2.03^{c}
TS45-5	0.44	0.44	0.44	1281.75 ± 3.51^{d}
TS45-10	0.43	0.43	0.43	1263.04 ± 2.03^{e}
TS45-15	0.42	0.43	0.43	1243.16 ± 3.51^{f}
TS60-5	0.41	0.41	0.41	1192.87 ± 2.03^{g}
TS60-10	0.41	0.41	0.41	1182.34 ± 2.03^{h}
TS60-15	0.40	0.40	0.40	1164.80 ± 4.05^{i}

The number with the different letters was a significant difference from each other ($p < 0.05$). **Pasteurized**: pasteurized beras kencur drink 100^{o}C for 2 min, **TS**: Thermosonication beras kencur drink, **TS30-5**: 30^{o}C for 5 min, **TS30-10**: 30^{o}C for 10 min, **TS30-5**: 30^{o}C for 15 min,

TS45-5: 45^{o}C for 5 min, **TS45-10**: 45^{o}C for 10 min, **TS45-15**: 45^{o}C for 15 min, **TS60-5**: 60^{o}C for 5 min, **TS60-10**: 60^{o}C for 10 min, **TS60-15**: 60^{o}C for 15 min.

The results of thermosonication treatment on FRAP value of beras kencur drink are mentioned in Table 1. There was a statistically significant difference ($p < 0.05$) in antioxidant activity. We found that the thermosonication treatment could maintain the FRAP value of beras kencur drink. The FRAP value of beras kencur drink treated with thermosonication ranged from 1164.80 – 1323.86 mgAA/L, while the FRAP value of beras kencur drink untreated and treated with pasteurization were 1300.47 and 902.81 mgAA/L, respectively.

Based on Table 1. beras kencur drink treated with a thermosonication temperature of 30 ^{o}C in all treatments of TS30-5, TS30-10 and TS30-15 increased the antioxidant activity of beras kencur drink compared with untreated samples. Phenolic compounds are one group of antioxidants that have many biological effects. Other studies showed that there was a positive correlation among the phenolic compound and antioxidant activity in the thermosonication treatment juice (Aadil *et al.,* 2013). Increasing the antioxidant activity could be attributed to the release of bioactive compounds in the sample due to the cell walls damaged due to pressure changes and shear forces by bubble collapse thereby enhancing the availability of these compounds (Manzoor *et al.,* 2021).

Based on the results, the higher temperature and time caused by thermosonication the antioxidant activity of the kencur rice drink was decreased. This decrease happened on beras kencur drink treated with thermosonication temperatures of 45 ^{o}C and 60 ^{o}C (TS45 and TS60) in all treatments. The decrease in antioxidant activity occurred as a result of several secondary antioxidants contained in the rice drink reacting with free radicals produced during the thermosonication process. Cansino *et al.* (2013) stated that phenolic compounds as secondary antioxidants can react as metal chelating agents by forming bonds with oxidized metal ions to stabilize them and reduce the redox potential. According to (Cullen, *et al.,* 2012) during the thermosonication process cavitation occurs which causes the sonolysis of water molecules that produce free radicals (H•, O•, OH•, HO_2•). The free radicals formed bind to bioactive components that act as antioxidants in the sample, thereby reducing antioxidant activity in the thermosonication treatment samples. Even though the result of thermosonication treatment samples was decreased, this decrease was smaller than the antioxidant activity in the pasteurized treatment sample.

2. Color

Color is a visual indicator of beras kencur drinks that directly affect the acceptance or rejection criteria of consumers. Color could give clues about chemical changes in food such as browning or caramelization. Table 2. showed the changes in the color value of beras kencur drink in various treatments.

Table 2. The color coordinates of beras kencur drink

Sample	Color values			TCD (ΔE)
	L*	a*	b*	
Untreated	62.90 ± 0.40^{a}	0.37 ± 0.15^{e}	20.38 ± 0.64^{d}	-
Pasteurized	48.49 ± 0.45^{f}	2.33 ± 0.20^{a}	25.07 ± 0.85^{a}	15.29 ± 0.41^{a}
TS30-5	62.58 ± 0.69^{ab}	0.42 ± 0.15^{e}	20.70 ± 0.40^{d}	0.50 ± 0.46^{g}
TS30-10	62.27 ± 0.69^{ab}	0.49 ± 0.11^{e}	20.70 ± 0.40^{d}	0.74 ± 0.60^{g}
TS30-15	61.77 ± 0.95^{bc}	0.55 ± 0.10^{de}	20.77 ± 0.70^{d}	1.24 ± 0.03^{f}
TS45-5	61.72 ± 0.34^{bc}	0.69 ± 0.09^{d}	20.83 ± 0.45^{d}	1.33 ± 0.19^{f}
TS45-10	61.68 ± 0.43^{bc}	1.07 ± 0.07^{c}	21.23 ± 0.51^{cd}	1.65 ± 0.16^{f}
TS45-15	61.28 ± 0.26^{c}	1.12 ± 0.08^{bc}	21.87 ± 0.57^{bc}	2.33 ± 0.16^{e}
TS60-5	60.13 ± 0.07^{d}	1.18 ± 0.02^{bc}	21.97 ± 0.31^{bc}	3.31 ± 0.12^{d}
TS60-10	59.67 ± 0.27^{d}	1.29 ± 0.02^{b}	22.27 ± 0.32^{b}	3.87 ± 0.20^{c}

TS60-15	58.16 ± 0.28^{e}	1.30 ± 0.11^{b}	22.53 ± 0.51^{b}	5.29 ± 0.07^{b}

The number with the different letters was a significant difference from each other ($p < 0.05$). **TCD:**Total Color Difference, **Pasteurized**: pasteurized beras kencur drink 100^{o}C for 2 min, **TS**: Thermosonicated beras kencur drink, **TS30-5**: 30^{o}C for 5 min, **TS30-10**: 30^{o}C for 10 min, **TS30-5**: 30^{o}C for 15 min, **TS45-5**: 45^{o}C for 5 min, **TS45-10**: 45^{o}C for 10 min, **TS45-15**: 45^{o}C for 15 min, **TS60-5**: 60^{o}C for 5 min, **TS60-10**: 60^{o}C for 10 min, **TS60-15**: 60^{o}C for 15 min.

Results revealed significant differences ($p < 0.05$) in the color values of beras kencur drink treated with thermosonication as compared to control untreated and pasteurized beras kencur drink. In this study, we found that increasing the temperature and time of thermosonication treatment caused the L* (lightness) was decreased while the a* (redness) and b* (yellowness) increased. The color values of beras kencur drink treated with thermosonication ranged from 58.16 – 62.58 for L*, 0,42 – 1.30 for a* and 20,70 – 22.53 for b*. While the TS60-15 treatment indicated the highest color value on a* and b* and the lowest value on L*. Color values of untreated beras kencur drink were 62.90 for L*, 0.37 for a*, 20.38 for b*, and the color values of pasteurized beras kencur drink were 48.49 for L*, 2.33 for a* and 25.07 for b*. The previous result reported by Abid *et al.* (2014); Jasmi *et al.* (2020); Jabbar *et al.* (2015), exhibited significant differences in the colors of the thermosonic treated liquid samples.

Based on Table 2. the result of total color difference (TCD) of beras kencur drink treated with thermosonication and pasteurization processing as compared to the control untreated was significant differences ($p < 0.05$). The TS30-5 sample had the lowest TCD (0.50) while the pasteurized treatment had the highest TCD (15.29). An increase in temperature and time of the thermosonic processing causes an increasing TCD of beras kencur drinks. The increasing value of TCD has been a result of the Maillard reaction (non-enzymatic browning) *(Manzoor et al.,* 2021). Non-enzymatic browning occurs at high temperatures and causes the sucrose molecule to undergo inversion, this causes browning and affects the color of the beras kencur drink (Akbar & Murtini, 2018). Similar results have also been reported by Abid *et al.* (2014); Jasmi *et al.* (2020); Jabbar *et al.* (2015), informing a significant difference in the TCD of the liquid sample after the thermosonic treatment.

Although thermosonication treatments showed significant differences in the color values and TCD values of beras kencur drink, these differences could not be perceived by the naked eyes. However, visually, the color of pasteurized beras kencur drink could be seen as the difference when compared to the thermosonication and untreated beras kencur drink. This result was similar to the statement of Khandpur and Gogate (2015).

CONCLUSIONS

In this study, known that the beras kencur drink thermosonication as a way to improve its quality as an alternative to the pasteurization process. The result shown that antioxidant activity and the color of beras kencur drink have been influenced by the temperature and time treatment. The thermosonication treatment at lower temperature levels can improve the antioxidant activity and progressively decreased with increasing temperature and treatment time, however the retention of these compounds is better than the pasteurization treatment. The color of beras kencur drink was treated with thermosonication indicates that, there was a statistically significant difference, but these differences could not be perceived by the naked eyes.

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