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The effect of hydrocolloid on stability of Papaya-Pineapple jelly drink during storage

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Abstract. Present study evaluated the effect of hydrocolloid type on the stability of antioxidant activity of papaya-pineapple jelly drink during storage. The antioxidant content of the jelly drink was also evaluated. The research had been performed using randomized block design with two factors, i.e.: types of hydrocolloid (agar and carrageenan) and storage times (0; 1; 2; and 3 weeks). Jelly drink was stored at 7°C in refrigerator. The analysis carried out were total phenolic content, ferric thiocyanate (FTC) and thiobarbituric acid (TBA) tests. The result showed that increasing of storaged resulted in decreasing of total phenolic content, ferric thiocyanate and thiobarbituric acid.

1. Introduction

Patients with degenerative diseases have recently been increasing due to the presence of free radicals. Free radicals are unstable and highly reactive molecules. They contain one unpaired electron in its outer orbital so that to achieve stability, free radicals react with surrounding molecules to obtain electron pairs [1]. The prevention of damage to the body because of free radical can be controlled with antioxidants. Antioxidants are compounds that can neutralize or reduce free radicals, and inhibit oxidation of body cells, so that they can prevent or reduce cell damage. Free radicals are atoms or molecules that have one or more unpaired electrons that can attract electrons from other compounds to form new free radicals and cause chain reactions. Free radical chain reactions can damage the macro molecules that make up cells such as DNA, proteins, carbohydrates, and lipids. Free radicals attack important macromolecules leading to cell damage and homeostatic [2].

Antioxidants are naturally present in plants. Antioxidants in plants in general are phenolic compounds. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, they have a metal chelation potential [3].

Papaya and pineapple are fruits that have the potential for antioxidants, because they contain phenol and vitamin C. Pineapple papaya jelly drink is one of the diversification of papaya and pineapple processing with the addition of hydrocolloids for gel formation. Agar and carrageenan are hydrocolloid compounds extracted from seaweed that contains high enough antioxidants. The antioxidant content in seaweed is mainly in the form of polyphenol [4].

Studies on content and antioxidant activity of pineapple papaya jelly drink with the addition of agar and carrageenan as hydrocolloids during cold storage have so far not been widely published. The

stability of polyphenols during storage can be affected by several external factors, such as air, storage temperature, and light. Total phenol content of green tea drinks with the addition of stevia decreased during cold storage [5]. Prolonged storage promotes both chemical and enzymatic oxidation of phenolic compounds, contributing to its reduction [6]. In this study, an evaluation of content and stability of antioxidant in pineapple papaya jelly drink was carried out with the addition of agar and carrageenan during cold storage.

2. Materials and methods

2.1. Material

Materials used in this study were papaya, pineapple, agar, carrageenan, sugar, citric acid, aquades, ethanol, Na2CO3, oleic acid, phosphate buffer, ammonium thiocyanate, ferrous chloride, trichloro acetic acid and thiobarbiturate acid. The equipment used were analytical balance, stainless steel blade, pan, stainless steel spoon, vessel for cooking, spoon stirrer, a washcloth, a stove, a plastic cup jelly drinks, and others. Tool to analyze is the UV-VIS spectrophotometer, analytical balance, Erlenmeyer, measuring cups, beaker glass, flask, pipette, etc.

2.2. Jelly drink processing

Pineapple papaya jelly drink was made by peeling and weighing the fruit, then the peeled fruits were blanched (papaya for 3 minutes and pineapple for 4 minutes). The fruit was then crushed using blender and filtered to obtain fruit juice. Fruit juice mixed with water (1: 4 w / v). The mixture was added with sugar (10% w / v), citric acid (0.25% w / v) and hydrocolloid (0.3% w/v). Hydrocolloids used were agar and carrageenan. The mixture was cooked to a boil. The jelly drinks were packed in plastic cups and closed using a cup sealer machine. Jelly drinks were then stored in the refrigerator (7oC) for 0; 1; 2; and 3 weeks.

2.3. Determination of total phenolic content

The total phenolic compounds in jelly drink were measured as gallic acid equivalents using the Folin-Ciocalteau's phenol reagent (FC reagent) according to the method described by [7] with some modifications. As much as 7.5 g of jelly drink was dissolved in 10 ml of 95% ethanol and filtered with filter paper. Briefly, 1 ml of extract solution was mixed with 45 ml of distilled water. One milliliter of Folin-Ciocalteu reagent was added and the content of the flask and mixed thoroughly. After 3 min 3 ml of Na2CO3 was added then the mixture was allowed to stand for 2 h. The absorbance was measured at 760 nm. Sample data compared with standard curve with gallic acid. Results were expressed in mg / g equivalent to gallic acid.

2.4. Determination of antioxidant activity test using FTC (ferric thiocyanate) method.

The FTC method were measured according to described by [8] with some modifications. Jelly drink extracts (4 mg) and standards (4 mg of vitamin E) were mixed with 4 ml of absolute ethanol, 4.1 ml of 2.52% linoleic acid in absolute ethanol, 8 ml of 0.02 M phosphate buffer (pH 7.0) and 3.9 ml of distilled water. The mixture was placed at 40°C (0.1 ml) and was then mixed with 9.7 ml of 75% (v/v), ethanol and 0.1 ml 30% ammonium thiocyanate. Three minutes after adding ferrous chloride (0.1 ml of 2×10-2 M ferrous chloride), the absorbance was measured at 500 nm in a spectrophotometer. This step was repeated every 24 h until the control reached its maximal absorbance value. The mixture without added sample was used as a control. The inhibition of lipid peroxidation (%) was estimated by the following formula:

$$\% \text{ Inhibition} = 100 - ((A1 - A0) \times 100).$$

where A0 is the absorbance of the control and A1 is the absorbance of the sample extracts

2.5. Determination of antioxidant activity test using Thiobarbituric acid (TBA) method [8]
Jelly drink extracts (2 ml) and standard solutions (2 ml) on the final day (day of maximal absorbance value) of the FTC assay were added to 1 ml of 20% aqueous trichloroacetic acid and 2 ml of 0.67% aqueous thiobarbituric acid. After boiling for 10 min, the samples were cooled. The tubes were centrifuged at 3,000 rpm for 30 min. Absorbance of the supernatant was evaluated at 532 nm in a spectrophotometer. The antioxidant activity was calculated by percentage of inhibition in this method as follows:

$$\% \text{ Inhibition} = 100 - [(A1 - A0) \times 100]$$

Where A0 is the absorbance of the control and A1 is the absorbance of the sample extracts

2.6. Research design
The study design was Randomized Block Design (RBD) with 2 treatments and 3 replications. The treatment in this study were hydrocolloid types: agar, carrageenan and storage times: 0; 1; 2; 3 weeks. The variables tested was analyzed using analysis of variance (ANOVA). If the analysis showed significant influence, it was followed by the DMRT (Duncan multiple-range test) with a 95% confidence interval

3. Results and discussion

3.1. Antioxidant content
Measurement of antioxidant levels is done by measuring the total phenol content. Analysis of total phenolic content was carried out to determine the potential of pineapple papaya jelly drink as a counteractant to free radicals and singlet oxygen stabilizer. The human body produces antioxidant compounds, but the amount is often not enough to neutralize free radicals that enter the body. Chemical components that act as antioxidants are phenolic and polyphenolic group compounds. The compounds of this group are widely found in nature [9]. The scavenging ability of the phenolics and flavonoids are mainly due to the presence of hydroxyl groups [10].

The total phenol content of jelly drink on the use of different types of hydrocolloid are shown in Figure 1.

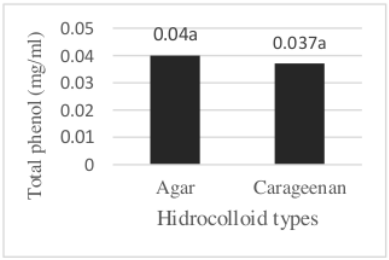

Figure 1. Total phenolic content of jelly drink in variations hydrocolloid type.

The type of hydrocolloid had no effect on the total phenol content of jelly dink. The total phenol content of jelly drink with hydrocolloid agar was 0.04 mg / ml and carrageenan 0.037 mg / ml. The hydrocolloids in jelly drinks are thought to come from the basic ingredients used (papaya and pineapple) as well as from hydrocolloids. Papaya containing vitamin C also contains phenols which act as antioxidants [11]. Phenolic compounds are able to ward off free radicals or act as antioxidants. Total phenolic content of agar derived from Sargassum horneri was 0.41 ± 0.01 mg GAE / g dry extract and Sargassum thunbergii was 0.29 ± 0.01 mg GAE / g dry extract [12]. Different types of seaweed produce different levels of total phenol. The total phenol content of the research results was smaller than the reference because hydrocolloid was only used as much as 0.3%. Low levels of hydrocolloid are thought

to cause total phenol levels which are not significantly different. The phenol content of papaya and pineapple is thought to increasing of jelly drink total phenol, enhance of jelly drink antioxidant content.

During the storage of jelly drinks, there was a decrease in total phenol levels. The total phenol content of jelly drinks during storage is shown in Figure 2.

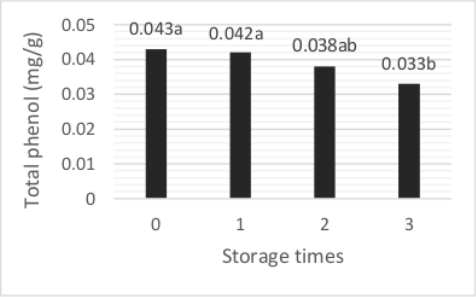

Figure 2. Total phenol content of pineapple papaya jelly drink during cold storage.

Storage of jelly drinks for 3 weeks caused a decrease in total phenol from 0.043 mg/g to 0.033 mg/g or a decrease of 23.26%. During storage, it is suspected that polymerization and degradation reactions occur which cause a decrease in the total phenol content. The stability of polyphenols during storage can be influenced by several external factors, such as air, storage temperature, and light [13]. Total phenol content of green tea drinks with the addition of stevia has decreased during cold storage [5]. Prolonged storage promotes both chemical and enzymatic oxidation of phenolic compounds, contributing to its reduction [6].

3.2. Antioxidant activity of jelly drinks

Jelly drink antioxidant activity testing was carried out using the FTC and TBA methods. The antioxidant activity of bioactive compounds is related to their structure and concentration in the plant food. In turn, the concentration of these bioactive compounds is largely influenced by genetic factors, ripeness degree, environmental and processing, storage time and packaging method [6]. The FTC and TBA tests measuring antioxidant activity based on the inhibition of fat peroxidation reactions. FTC is used to measure the initial amount of peroxide from fat peroxidation, while the TBA method is to measure the free radicals generated after fat peroxidation. The free radicals inhibited by the FTC and TBA methods were peroxide and malonaldehyde compounds.

The inhibitory activity of peroxide formation which was carried out using the FTC method at variations of hydrocolloids shown in Figure 3.

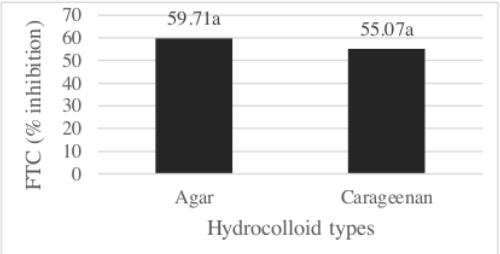

Figure 3. Inhibitory activity of peroxide formation using the FTC method in variations of hydrocolloid type.

Figure 3 shows that the type of hydrocolloid had no significant effect on the inhibitory activity of peroxide formation. Presumed that the low concentration of hydrocolloids (0.3%) used in the manufacture of jelly drinks cause not significantly different. The inhibition of peroxide formation ranged from 55.07 to 59.71%, indicating high antioxidant activity. The antioxidant activity is classified as very high if it has an inhibitory activity of more than 90%; high antioxidant activity 50% - 90%; moderate antioxidant activity 20% -50%; low antioxidant activity of less than 20%; and showed no 0% antioxidant activity [11]. The antioxidant activity of jelly drinks is thought to be produced by phenol and vitamin C compounds derived from papaya, pineapple and hydrocolloids. Papaya has high peroxide inhibition activity. Pineapple contains phenols and has inhibitory activity in the DPPH test [14].

The results of the jelly drink antioxidant stability test measured using the FTC method showed a decrease during storage. The decrease in antioxidant activity of jelly drinks during storage is shown in Figure 4.

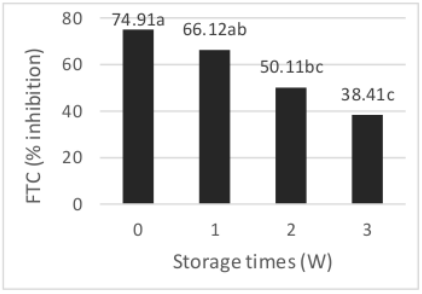

Figure 4. The activity of peroxide formation (FTC) inhibition of jelly drink during storage.

During storage there is a decrease in inhibition of peroxide formation. During 3 weeks of storage jelly drinks in the refrigerator (temperature 7°C), there was a decrease in the inhibition ability of peroxide formation by 48.73%. The total phenolic reduction during storage is suppose cause in decreasing antioxidant activity. The total phenolic levels are directly proportional to antioxidant activity [15]. The test results obtained indicate that there is a positive relationship between total phenol and antioxidant activity in reducing iron ions. Phenolic compounds have hydroxyl groups so they can donate electrons by reducing Fe^{3+} to Fe^{2+} [5]. The decrease in antioxidant activity during cold storage is also thought to be due to chemical changes, especially in the activity of compounds as antioxidants [5].

The inhibitory activity of peroxide formation carried out using the TBA method at variations of hydrocolloids is shown in Figure 5.

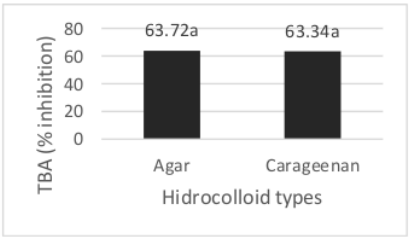

Figure 5. Inhibition activity of malonaldehyde formation using the TBA method on variations in hydrocolloids types.

Figure 5 shows that the type of hydrocolloid has no significant effect on the inhibitory activity of malonaldehyde formation. Malonaldehyde compounds are derivatives of peroxide compounds formed in the second stage of the fat peroxidation reaction. The use of low hydrocolloids (0.3%) in the

manufacture of jelly drinks is thought to cause malonaldehyde formation which is not significantly different. The inhibition of malonaldehyde formation ranged from 63.34 to 63.73%, indicating high antioxidant activity.

The results of the jelly drink antioxidant stability test measured using the TBA method (measuring the inhibition of malonaldehyde formation) showed decrease during storage. The decrease in antioxidant activity of jelly drinks during storage is shown in Figure 6.

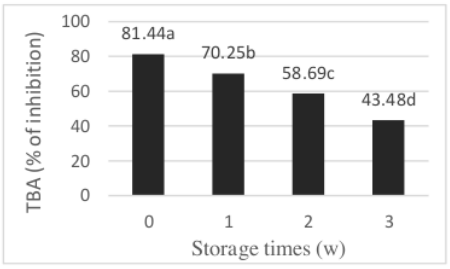

Figure 6. Inhibitory activity formation malonaldehyde of jelly drink during storage.

During storage, there is a decrease in inhibition of malonaldehyde formation. During 3 weeks of storing jelly drinks in the refrigerator (temperature 7°C), there was a decrease in the ability to inhibit aldehyde formation by 48.73%. The total phenolic reduction during storage is thought to have an effect on decreasing antioxidant activity.

The antioxidant activity detected by the TBA method was higher than that of FTC. The amount of peroxide formed in the early stages of lipid peroxidation is thought to be smaller than the number of peroxide derivatives formed in the second stage. In addition, the malonaldehyde are formed more stable over time [16].

4. Conclusion

Cold storage of jelly drink in a refrigerator at 7°C causes a decrease in antioxidant level and activity. Pineapple papaya jelly drink with the addition of agar and carrageenan has the potential to inhibit lipid peroxidation in initial stage (FTC) and second stage (TBA). The antioxidant activity of the TBA method is higher than the antioxidant activity of the FTC assay, therefore, the higher antioxidant activity found from the TBA method, indicated that the amount of peroxide in the secondary stage of lipid peroxidation was greater than the amount of peroxide in the initial stage

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