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# Study on the stability of antioxidant and anti α-glucosidase activities using soaking treatment in Okra (*Abelmoschus esculentus* L.) mucilage extraction

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**A B S T R A C T**

Okra fruit (*Abelmoschus esculentus* L.) especially its mucilaginous properties has been reported to have various health benefits include antioxidant and anti-diabetic properties. This research was aimed to investigate the stability of antioxidant and anti-α-glucosidase activities (as one of antidiabetic activities) of okra mucilage through several soaking treatments in term optimizing mucilage extraction as well. Okra fruit and its peel are used and soaked with water in several soaking conditions: time (1, 4, 8, and 12 h); temperature (room and refrigerator temperature); and ratio of fruit:water (1:3 and 1:6). Analysis of yield percentage, flavonoid content, free radical scavenging activity and anti α-glucosidase activity were observed. The highest antioxidant activity of the sample was found in the extracting methods of 12 h in refrigerator temperature with soaking ratio of 1:6 (897.26+19.67 mg/L) while for anti α-glucosidase activity was found in extracting methods of 12 h in refrigerator temperature with soaking ratio of 1:3 (525.92 ± 40.75 mg/L). The stability of antioxidant and anti α-glucosidase activities of okra mucilage might be influenced by the extracted flavonoid content and other active components.



**Capsule Summary:** Okra fruit *(Abelmoschus esculentus L.)* mucilaginous was studied at different soaking treatments and extracts showed considerable antioxidant and anti-α-glucosidase activities.

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## INTRODUCTION

Okra is the local Indonesia name of *Abelmoschus esculentus* L. or known in many west countries as lady's fingers or gumbo is commonly consumed as a healthy vegetable due to its rich medicinal value. Okra plant itself is now much cultivated throughout all over tropical, subtropical, and warm temperate regions of the world mainly for culinary purposes and still poorly studied (Lim, 2012; Maganha et al., 2010). In Indonesia, okra fruit has been planted well in some areas such as Bandung, and has been distributed to some groceries and markets. However, okra fruit is still barely known by people.

Duncan et al. (2012) stated that okra fruit has mucilaginous substances which mean it produces mucilage. Okra mucilage is believed to have ability in normalizing blood sugar of human body. Besides being cooked, people tend to soak chopped okra fruit into water and drink it instead of being boiled or eaten raw. Literature has stated that okra

fruit, especially its mucilaginous properties have various health benefits include antioxidant and anti-diabetic properties (Collins, 2010; Tomoda et al., 1989). However, there is no further study about soaking treatments on okra mucilage extraction toward antioxidant and anti-diabetic activity. Thus, this research is focus on the stability of antioxidant and anti α-glucosidase activities of okra mucilage through several soaking conditions in term optimizing mucilage extraction as well.

Okra *(Abelmoschus esculentus L.)*

## MATERIAL AND METHODS

### Chemical and reagents

Main material in this research is okra fruit (*Abelmoschus esculentus* L.) which is planted and harvested in Bandung, West Java. Materials used for analysis are DPPH (1,1-diphenyl-2-picryl-hydrazyl), methanol, $AlCl_3$ methanolic solution, ethanol, acetone, DMSO, glucosidase enzyme, phosphate buffer, bovine serum albumin, p-nitrophenyl α-D-glucopyranoside, and $Na_2CO_3$.

### Methods

Raw fresh okra fruits (*Abelmoschus esculentus* L.) were sorted manually which green color fruit was desired, then washed using tap water, seed were removed and sliced. Then 10 g of sliced okra fruit were weighed and soaked with water for certain conditions prior to analysis. There were four different soaking times applied: 1, 4, 8 and 12 h. Each soaking time applied two different temperatures (room temperature of 22-23 $^oC$ and refrigerator temperature of 4-5 $^oC$) and two ratios of okra fruit and water (1:3 and 1:6). According to Kulkarni *et al.,* (2002), mucilage is a water-soluble polysaccharide therefore in this research, water is used as a solvent. Then after being soaked, it was boiled (80-85 $^oC$) for 30 min, left undisturbed for 1 h at room temperature in order to complete release of the mucilage into water (Shah and Patel, 2010). After that, it was being filtered by using filter cloth. Then the filtered liquid was later called mucilage extract.

### Experiment design

The completely randomized three-factor factorial design with three replicates is used. The statistical model of the design is:

$$Y_{ijkl} = \mu + \tau_i + \beta_j + \gamma_k + (\tau\beta)_{ij} + (\tau\gamma)_{ik} + (\beta\gamma)_{jk} + (\tau\beta\gamma)_{ijk} + \varepsilon_{ijkl}$$

Where,

$Y_{ijkl}$ = observation value at level one, with factor of soaking time for extraction on level i, factor of soaking temperature for extraction on level j, factor of soaking ratio for extractionon level k, and replication on level l

$\mu$ = actual mean value

$\tau_i$ = effect of soaking time for extraction on level i

$\beta_j$ = effect of soaking temperature for extraction on level j

$\gamma_k$ = effect of soaking ratio for extraction on level k

$(\tau\beta)_{ij}$ = effect of interaction between soaking time for extraction on level i and soaking temperature for extraction on level j

$(\tau\gamma)_{ik}$ = effect of interaction between soaking time for extraction on level i and soaking ratio for extraction on level k

$(\beta\gamma)_{jk}$ = effect of interaction between soaking temperature for extraction on level j and soaking ratio for extraction on level k

$(\tau\beta\gamma)_{ijk}$ = effect of interaction among soaking time for extraction on level i, soaking temperature for extraction on level j and soaking ratio for extraction on level k

$\varepsilon_{ijkl}$ = error factor

The null hypothesis ($H_0$) for this stage are:

1) There is no significant effect of soaking time, soaking temperature, and soaking ratio for extraction towards the antioxidant and anti-diabetic activity.

2) There is also no interaction between soaking time for extraction and soaking temperature for extraction, soaking time for extraction and soaking ratio for extraction, soaking temperature for extraction and soaking ratio for extraction towards the antioxidant and anti-diabetic activity.

3) There is no interaction among the soaking time, soaking temperature, and soaking ratio for extraction towards the antioxidant and anti-diabetic activity.

The alternative hypothesis ($H_1$) are:

1) There is significant effect of soaking time, soaking temperature, and soaking ratio for extraction towards the antioxidant and anti-diabetic activity.

2) There is also interaction between soaking time for extraction and soaking temperature for extraction, soaking time for extraction and soaking ratio for extraction, soaking temperature for extraction and soaking ratio for extraction towards the antioxidant and anti-diabetic activity.

3) There is interaction among the soaking time, soaking temperature, and soaking ratio for extraction towards the antioxidant and anti-diabetic activity.

### Yield determination

**Table 1:** Experimental design used for experimentation

| Soaking time (A) | Soaking temperature (B) | | | |
|---|---|---|---|---|
| | Room (B1) | | Refrigerator (B2) | |
| | Ratio of Okra fruit and water | | | |
| | 1:3 (C1) | 1:6 (C2) | 1:3 (C1) | 1:6 (C2) |
| 1 hour (A1) | A1B1C1 | A1B1C2 | A1B2C1 | A1B2C2 |
| 4 hours (A2) | A2B1C1 | A2B1C2 | A2B2C1 | A2B2C2 |
| 8 hours (A3) | A3B1C1 | A3B1C2 | A3B2C1 | A3B2C2 |
| 12 hours (A4) | A4B1C1 | A4B1C2 | A4B2C1 | A4B2C2 |

The yield percentage of extraction was obtained from the ratio of weight (w) of starting material (SM, dry basis) and the end product (extract, dry basis) times 100%. The formula of extraction yield calculation is shown in Eq. 1. Where, E = extracts and MC = moisture contents (Wang et al., 2011).

$$Yield\ (\%) = \left\{\frac{E_W-(100\%-E_{MC})}{SM_W-(100\%-SM_{MC})}\right\} x\ 100 \qquad (1)$$

**Analysis of flavonoid content using Dowd method**

The total flavonoid content of sample was determined using Dowd method. Firstly, 2 mL of sample is mixed with 2 ml of 2 % $AlCl_3$ methanolic solution until homogenous. Then the solution was allowed to stand for 10 minutes prior to absorbance measurement using spectrophotometer at 415 nm against the blank. The blank solution was prepared by mixing 2 mL of methanol and 2 mL of diluted sample, without the addition of $AlCl_3$ solution. Finally, a standard curve of quercetin must be established and the total flavonoid content was expressed as mg quercetin equivalent/ L of sample (Arvouet-Grand et al., 1994).

**Analysis of free radical scavenging activity**

Total solid of each sample was calculated and diluted in methanol into certain concentrations: 100 ppm, 500 ppm, 750 ppm and 1000 ppm. Then DPPH was diluted in methanol to reach 0.2 mM solution. After that, about 1 mL DPPH solution was added with 0.2 mL of sample and the absorbance was measured at 517nm. The control solution was prepared by mixing 1 mL of DPPH solution and 0.2 mL of methanol, while for blank solution is prepared using 1.2 mL methanol. Finally, the process was performed at 30 minutes and then the free radical scavenging activity was determined as percentage of scavenging effect (Hwang, 2009).

**Analysis of α-glucosidase inhibitory activity**

The mixture used was made from reaction system of 250 µL of 20 mM p-nitrophenyl-α-D glucopyranose, 490 µL of 100 mM phosphate buffer, and 10 µL of sample in DMSO or DMSO as a blank. The mixture then was incubated for 37 °C for 5 min. 250 µL of enzyme was added into the mixture into the positive control and sample. 250 µL of phosphate buffer was added into negative control and blank. Then the mixture was incubated for 37 °C for 15 min. The inhibition reaction was stopped by 1000 µL of $Na_2CO_3$ addition to the each mixture (Sabhita, 2012).

Then, the calculation for inhibition activity was calculated as shown in Eq. 2.

$$Inhibition\ (\%) = \left\{\frac{C-(S_1-S_0)}{C}\right\} x\ 100 \qquad (2)$$

Where, C is the absorbance of positive control, $S_1$ is the absorbance of the sample; $S_0$ is absorbance of negative control of sample. Then, the $IC_{50}$ value is determined as the concentration of α-glucosidase inhibitor that inhibits 50 % of α-glucosidase activity.

**RESULTS AND DISCUSSION**

**Taxonomical verification**

Okra fruit that is used in this research is *Abelmoschus esculentus* (L.) Moench, which has been identified by Indonesian Institute of Sciences (LIPI), based on the statistical analysis of yield percentage of mucilage extracted; there was no interaction between soaking time, temperature and ratio. However there was interaction between soaking time and temperature, soaking time and ratio, also soaking temperature and ratio at $p \leq 0.05$.

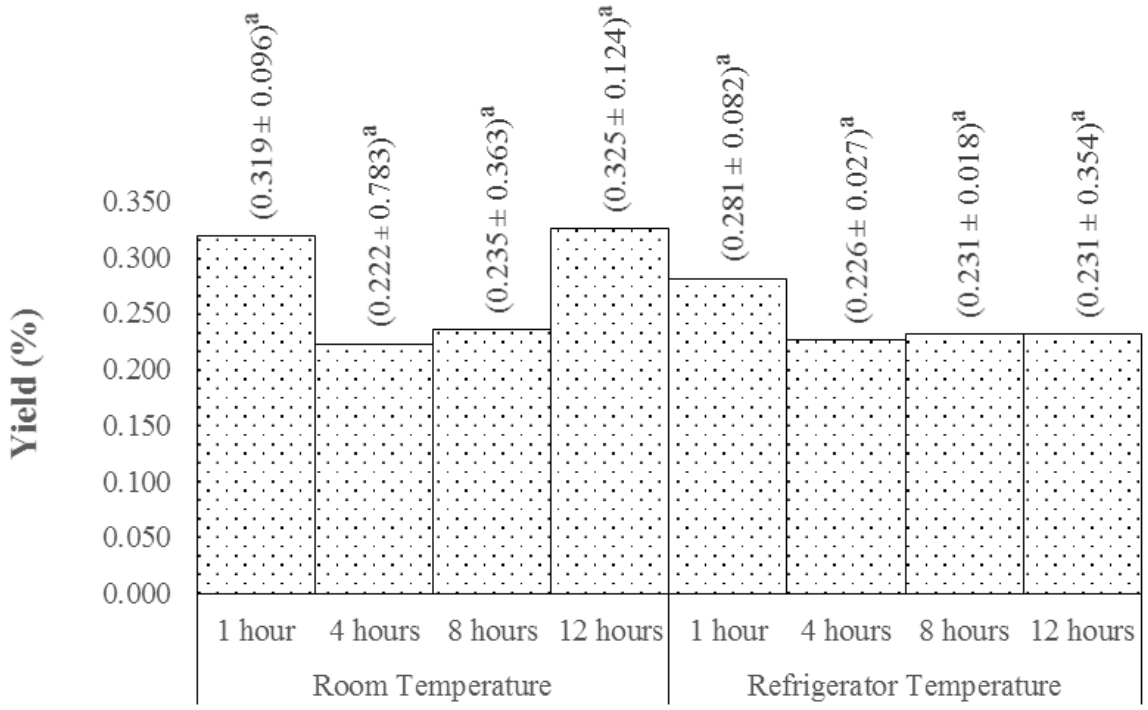


**Fig. 1:** Yield (%) of mucilage extracted from interaction between soaking time and temperature (Note: Different alphabets indicate significant difference at 95% level).

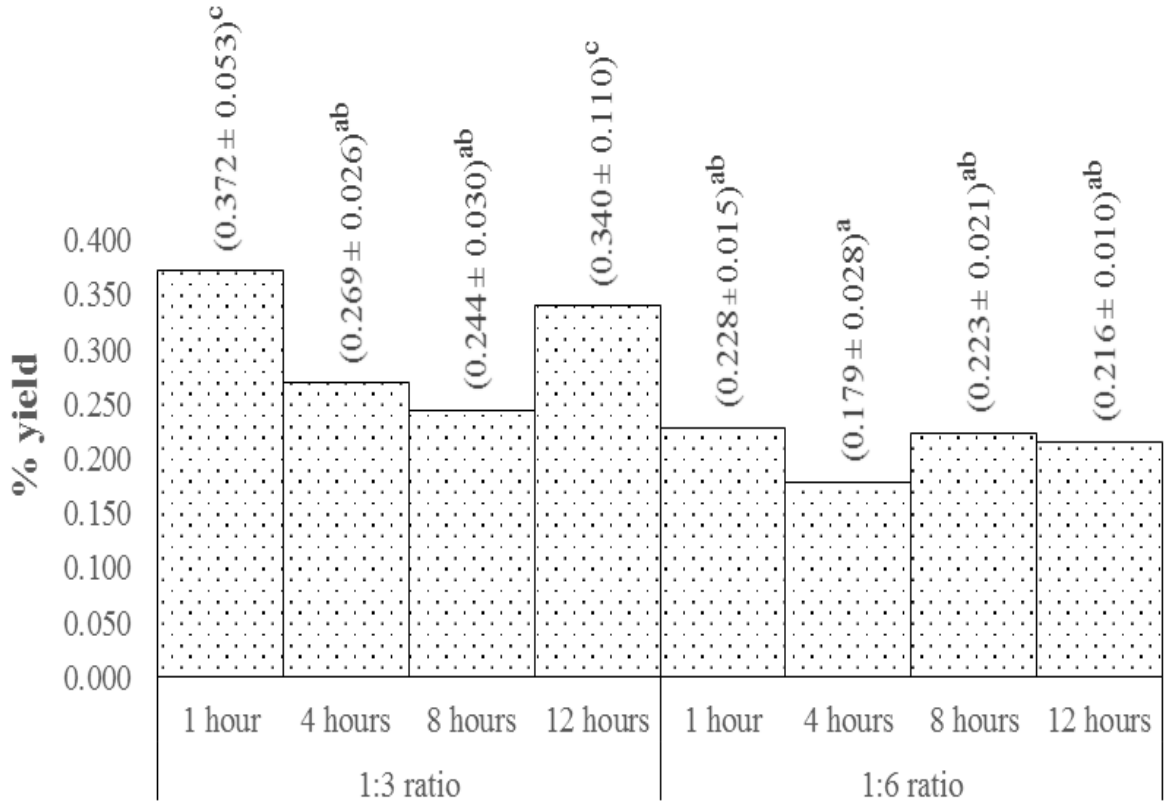


**Fig. 2:** Yield (%) of mucilage extracted from interaction between soaking time and ratio (Different alphabets indicate significant difference at 95% level).

**Interaction between soaking time and temperature**

In this experiment, although there was interaction between soaking time and temperature, it was not significantly different of yield percentage obtained from each sample under different soaking conditions at $p \leq 0.05$.

According to Islam (2008), extraction time influences the yield in which the longer extraction time will result in the degradation of plant materials thus producing low efficiency. However, in this experiment, the longer time extraction did not give more yield percentage of mucilage extracted. According to Tiwari *et al.,* (2013), the high temperature that was applied in the extraction influences the yield of extraction which the higher temperature will break down the cellular materials that gives result in the increasing yield of extracted compounds. But, each sample from different soaking time and temperature in this experiment was then being heated (80-85 $^{o}$C) for 30 min and left undisturbed for 1 h to release more mucilage, so due to this reason, the yield percentage of okra mucilage obtained was not significantly different between the samples.

**Interaction between soaking time and ratio**

The interaction between soaking time and ratio of yield percentage was significantly different at $p \leq 0.05$. The best yield percentage of okra mucilage extracted was obtained from sample which was soaked for 12 h with 1:3 ratio (0.340 ± 0.110 %) and the one that was soaked for 1 h with 1:3 ratio (0.372 ± 0.053 %). From this experiment, it can be seen that longer time did not give higher yield percentage. Actually longer time of soaking influences the yield percentage, which according to Islam (2008), extraction time influences the yield in which the longer extraction time will result in the degradation of plant materials thus producing low efficiency. However time is not the only one factor concerned in this experiment, so that other factors might lead to these unexpected results. Not only that, the lower soaking ratio also gave higher yield percentage of okra mucilage extracted.

**Interaction between soaking temperature and ratio**

Based on this experiment, there was interaction between soaking temperature and ratio, and it was significantly different of yield percentage obtained from each sample under different soaking conditions at $p \leq 0.05$. The best yield percentage of okra mucilage extracted was obtained from the sample that was soaked in room temperature with 1:3 soaking ratio (0.344 ± 0.082 %). In this experiment, the higher temperature of soaking and the lower soaking ratio gave the best yield percentage of okra mucilage extracted. It may be happened due to higher temperature will break down the cellular materials that gives result in the increasing yield of extracted compounds (Tiwari *et al.,* 2013). So the result of yield percentage of okra mucilage extracted in this experiment shows that higher temperature of soaking indeed increases the yield percentage. Not only that, the lower soaking ratio also gave higher yield percentage of okra mucilage extracted.

**Effect of soaking time, temperature, and ratio to flavonoid content**

According to Khomsug et al. (2010), okra fruit contains flavonoids that were identified as procyanidin B2, procyanidin B1, rutin, quercetin, catechin and epicatechin. Based on the statistical analysis of flavonoid content, there was a significant interaction of soaking time, soaking temperature, and soaking ratio toward flavonoid content as well as there was a significant effect of each soaking conditions at $p \leq 0.05$.

The highest flavonoid content is shown by sample which was obtained from 12 h soaking of okra fruit in room temperature (22-23 $^{o}$C) with soaking ratio of 1:3 (72.17±0.36 mg quercetin/L) and followed by sample which was obtained from 12 h soaking of okra fruit in refrigerator temperature (4-5 $^{o}$C) with soaking ratio of 1:3 (61.34 ± 0.22 mg quercetin/L). The lowest flavonoid content is shown by sample which was obtained from 12 h soaking in refrigerator temperature with 1:6 soaking ratio (32.24 ± 0.37 mg quercetin/L). This sample shows a significant difference with other samples.

In this experiment, as can be seen in Figure 4, the longer soaking time especially 8 and 12 h give higher flavonoid content than 1 and 4 h soaking time, this happens whether in room temperature or refrigerator temperature. Lower soaking temperature also shows tendency of yielding lower content of flavonoid compare with the one that is soaked in room temperature. This may be happened because the flavonols may decrease throughout prolong low temperature applied (Cordenunsi et al., 2005). Soaking ratio

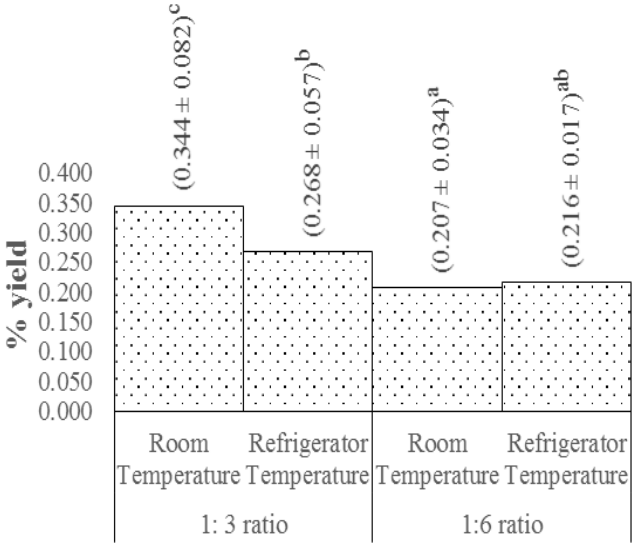


**Fig. 3:** Yield (%) of mucilage extracted from interaction between soaking temperature and ratio (Different alphabets indicate significant difference at 95% level).

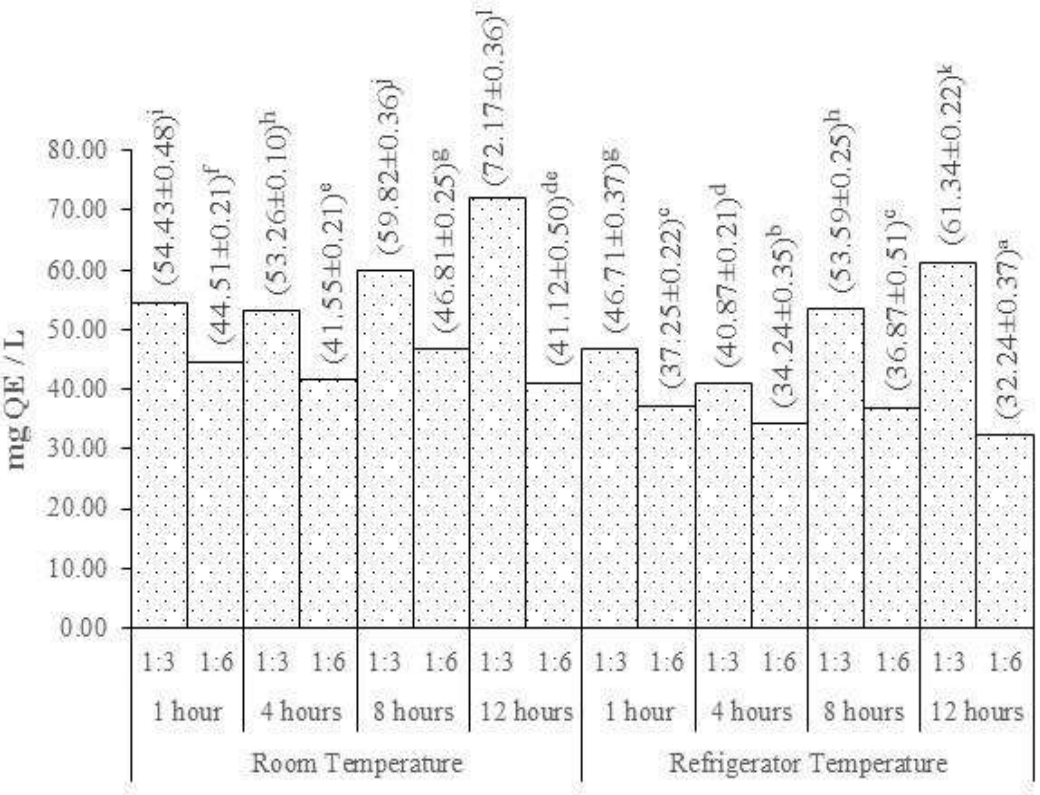


**Fig. 4:** Flavonoid Content (mg Quercetin/L) from different soaking conditions (Different alphabets indicate significant difference at 95% level).

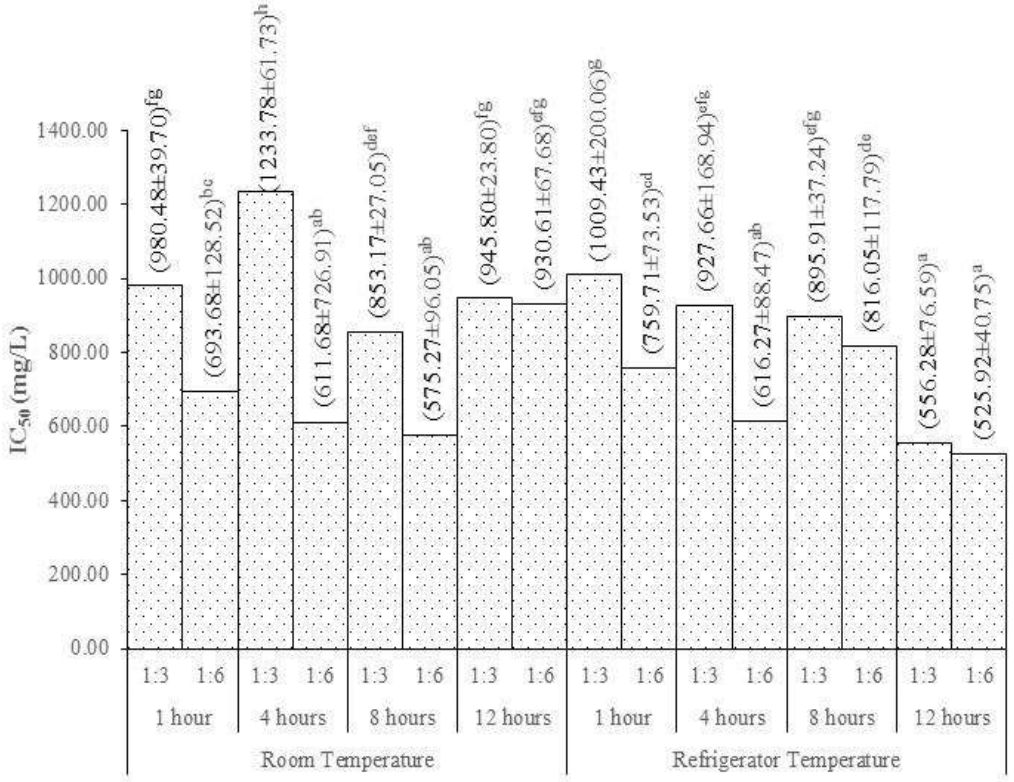


**Fig. 5:** $IC_{50}$ value of antioxidant activity of okra mucilage from different soaking treatments (Different alphabets indicate significant difference at 95% level).

of 1:3 also seems to give higher yield compare with the 1:6 ratio.

**Effect of soaking time, temperature, and ratio towards antioxidant activity**

In this experiment, the result of antioxidant activity of different soaking conditions of okra mucilage extractions are shown by the percentages of inhibition activity. Then the $IC_{50}$ value is calculated, which represent the amount of sample needed to reduce 50% of DPPH radicals. A good antioxidant activity is shown by low $IC_{50}$, which the lower $IC_{50}$ value is a better antioxidant activity. As can be seen from the statistical analysis of antioxidant activity, there was a significant interaction of soaking time, soaking temperature, and soaking ratio toward antioxidant activity as well as there was a significant effect of each soaking conditions at $p \leq 0.05$.

The highest antioxidant activity is shown by sample which was obtained from 12 h soaking of okra fruit in refrigerator temperature (4-5 $^oC$) with soaking ratio of 1:6 (525.92 ± 40.75 mg/L) similar with soaking ratio 1:3 same temperature (556.28 ± 76.59 mg/L). The use of shorter soaking time in room temperature also gives similar antioxidant activity (4 and 8 h in room temperature). The lowest antioxidant activity is shown by sample which was obtained from 4 h soaking in room temperature with 1:3 soaking ratio (1233.78 ± 61.73 mg/L).

It can be inferred that higher temperature will resulted in the shorter time in extracting antioxidant components. However the extract will prone to lose its antioxidant activity if prolong soaking is continued as can be seen in Figure 4.5, the antioxidant activity is getting higher through longer soaking time while the sample which was obtained from soaking in room temperature shows getting higher at the first 8 h soaking but then decreasing prolong hours of soaking (12 h). According to Reblova (2012), one of the most important factors affecting the antioxidant activity is temperature, in which the higher temperature applied will decrease the antioxidant activity that means a decrease in the ability to react with free radicals. Moreover, according to Yanishlieva (2011), variations in temperature can also change the mechanism of action of some antioxidants. From the result of this experiment, it can be concluded that the kind of antioxidant components in mucilage extracted has a quite good potential in oxidation. Besides temperature, soaking ratio influences the activity of antioxidant, in which the higher soaking ratio results in lower antioxidant activity.

According to Khomsug et al. (2010), it has been reported that okra fruit has high amounts of total flavonoids and moderate amounts of total phenolics which both components are a good source of natural antioxidants. The flavonoid content of okra fruit which has biggest portion in phenolic compound, may affect the antioxidant activity of okra fruit. This conclusion is strengthened by the research which was done by Liao et al. (2012) that two flavonol glycosides that were found in okra fruit (*Abelmoschus esculentus* (L.) Moesch) have strong ability for scavenging DPPH. As mentioned before, the antioxidant activity of sample becomes lower due to higher temperature of soaking although Cordenunsi *et al.,* (2005), in his paper found that the presence of flavonols and total phenolic contents are stable in prolong low temperature storage. However, the antioxidant activity is loss.

The highest antioxidant activity obtained from different soaking conditions (525.92 mg/L) is still not as strong as antioxidant activity from ascorbic acid (8.56 mg/L). Whereas, according to Kasture and Wadodkar (2008), the stronger the antioxidant will inhibit oxidation for longer period of time. The reason ascorbic acid was used as a standard for comparison is because ascorbic acid is known as vitamin C that is trusted as an antioxidant. The mucilage extract in this experiment is still containing many other components which might explained the occurrence of low free radical scavenging activity.

**Effect of soaking time, temperature, and ratio towards stability of anti α-glucosidase activity**

In this experiment, anti α-glucosidase assay was performed as it is one of the methods of anti-diabetic or hypoglycemic activity. The result of anti α-glucosidase activity of different soaking treatments of okra mucilage extractions are shown by the percentages of inhibition activity $IC_{50}$ value. The $IC_{50}$ value is determined as the concentration of anti α-glucosidase that inhibits 50 % of α-glucosidase activity. A good α-glucosidase inhibitory activity is shown by low $IC_{50}$, which the lower $IC_{50}$ value is a better α-glucosidase inhibitory activity. As can be seen from the statistical analysis of anti α-glucosidase activity, there was a significant interaction of soaking time, soaking temperature, and soaking ratio toward anti α-glucosidase activity as well as there was a significant effect of each soaking conditions at $p \leq 0.05$, except interaction between soaking temperature and soaking ratio. The highest anti α-glucosidase activity is shown by sample which was obtained from 12 h soaking of okra fruit in refrigerator temperature with soaking ratio of 1:3 (897.26 ± 19.67 mg/L) as similar with the one was obtained from 12 h soaking time in room temperature with soaking ratio 1:3 (1019.33 ± 5.67 mg/L). The lowest anti α-glucosidase activity is shown by sample which was obtained from 1 h soaking in room temperature with 1:6 soaking ratio (2492.62 ± 151.97 mg/L).

In this experiment, the 12 h of soaking in either room or refrigerator temperature with either 1:3 or 1:6 ratio, gives the lowest $IC_{50}$ value of anti α-glucosidase activity if compared with other treated samples. It may be happened because the longer extraction time might also extract other active component that will also affect the α-glucosidase inhibitory activity. Previous study by Saha et al. (2011), aqueous extract of okra fruit (*Abelmoschus esculentus* L.) shows a qualitatively positive result of the presence of flavonoids and a quantitatively maximum effect of hypoglycemic activity towards adult albino wistar rats. According to Sharma et al. (2008) show that flavonoid content plays role in hypoglycemic activity or anti-diabetic activity, which quercetin as flavonols is one of the factors that

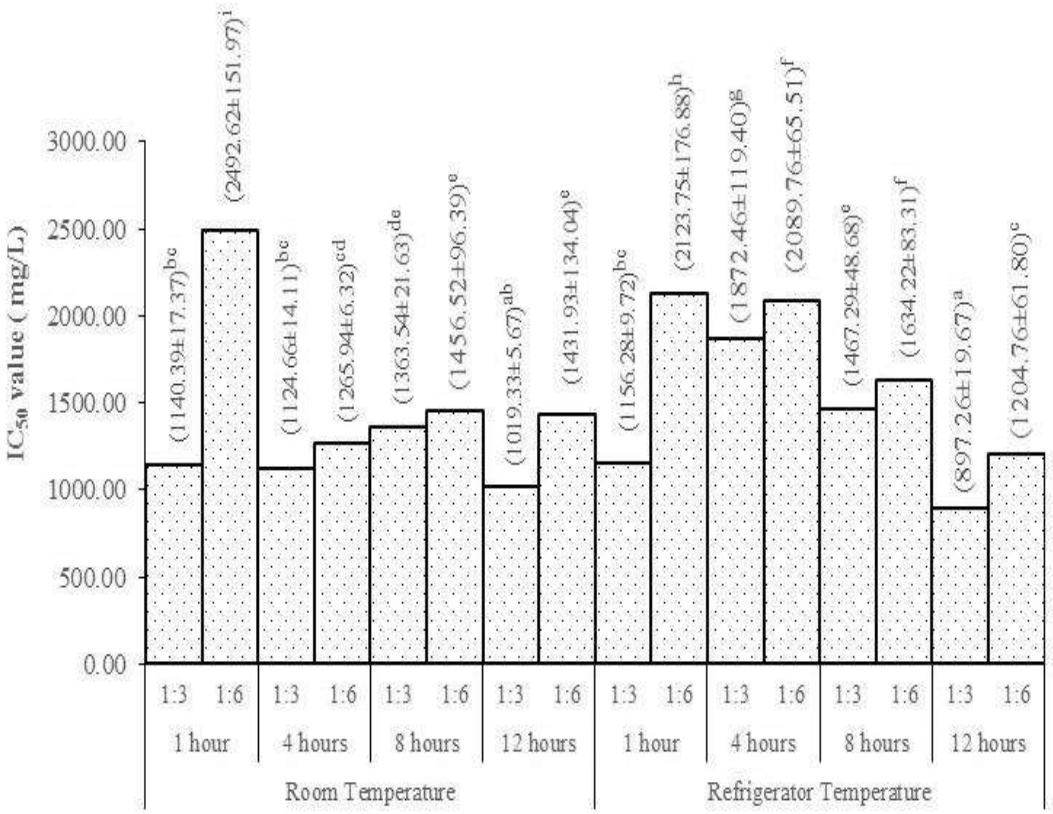


**Fig. 6:** $IC_{50}$ value of anti α-glucosidase activity of okra mucilage from different soaking treatments (Different alphabets indicate significant difference at 95% level)

induce insulin in pancreatic. Insulin itself acts in converting the excess glucose (simple carbohydrate) in human body into glycogen (complex carbohydrate) for storage (Freberg, 2010). So that, the result of anti α-glucosidase activity may be affected by the flavonoid content as well as antioxidant activity. Different soaking temperature however gives different result in α-glucosidase inhibitory activity. Mucilage extracted from soaking in room temperature gives lower $IC_{50}$ value and also higher anti α-glucosidase activity compare to the extract soaked in low temperature. The soaking ratio of 1:3 shows higher anti α-glucosidase activity than 1:6 soaking ratio.

However, the highest anti α-glucosidase activity obtained from different soaking conditions (897.26 mg/L) is still consider low compare to the anti α-glucosidase activity from acarbose (19.21 mg/L), which might explained by the impurities of the mucilage extract in this experiment. The presence of other components beside flavonoid content might affect its ability toward anti α-glucosidase activity. From statistical test, flavonoid content shows to have positive correlation with anti α-glucosidase activity with the Pearson's number of 0.633 in okra mucilage extracted. As what has been explained before, flavonoid content plays role in hypoglycemic activity (Sharma et al., 2008). However, the result of in this experiment, the correlation between flavonoid content and antioxidant activity is negative. It might be happened due to two reasons. The first was the heat applied in releasing more of mucilage from fresh fruit okra which the flavonoid content was indeed being extracted more, however the antioxidant activity might be decreased. The second was the unpurified or unconcentrated of the mucilage extracted which the water as the solvent used for extracting was not being evaporated to get the concentrated mucilage or pure mucilage. Not only that, there is also

**Table 2:** Correlation of flavonoid content, antioxidant and anti α-glucosidase activity

| | Flavonoid | Antioxidant Activity | Anti α-glucosidase Activity |
|---|---|---|---|
| Flavonoid | 1 | -.344** | .633** |
| Antioxidant_Activity | -.344* | 1 | -.065 |
| Antidiabetic_Activity | .633** | -.065 | 1 |

Note: * indicate correlation is significant at 95% level, ** indicate correlation is significant at 99% level

negative correlation between antioxidant and anti α-glucosidase activity. It might be happened because both of them play in different role in human body. According to Young (1998) and Sen (2000), antioxidant brings to a significantly decrease the adverse effects of reactive oxygen species, reactive nitrogen species, or both on normal physiological function in humans. Anti-diabetic itself plays role in decreasing glucose oxidation, non-enzymatic glycation of proteins and subsequent oxidative degradation of glycated proteins in which are responsible for the presence of oxygen free radicals formation in diabetes.

## CONCLUSIONS

In this experiment, okra mucilage gives positive result in exhibiting the antioxidant and anti α-glucosidase activities. Statistically, different soaking treatments (time, temperature and ratio) shows an interaction to the stability of the activities of each samples including the flavonoid content but not to the yield percentage of mucilage extracted. There are significant difference between soaking time and temperature; soaking time and ratio; soaking temperature and ratio toward the yield percentage, flavonoid content and antioxidant activity, except for the yield percentage and anti α-glucosidase activity in which there is no effect of soaking temperature and soaking ratio. According to the result, soaking in room temperature with 1:6 soaking ratio gives higher yield percentage of okra mucilage extracted if compared with others. The best antioxidant activity of okra mucilage extracted is found in the extracting methods of 12 h in refrigerator temperature of 4 $^{o}$C - 5 $^{o}$C with soaking ratio of 1:6 while for the α-glucosidase inhibitory

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