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# *Phytochemical Characteristics and Antioxidant Activity of Liang Tea at Different pH During Storage*

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**Abstract**

*Tea is a popular beverage consumed in Pontianak City, Indonesia. The ingredients of tea were consist of the leaves of D.chinensis, R.discolor, O.aristatus, P.amaryllifolius, heartwood of C.sappan and peels of A.vera with certain ratio. The study aims to find out the effect of storage time on phytochemical content and antioxidant activity of liang tea with different pH. The liang tea was prepared by hot brewing of the tea ingredients for 6 minutes and let it cooled down. The pH of tea adjusted to 4, 5, 6 and 6,5 by adding citric acid buffer and stored in plastic container and leave it at room temperature. The observed parameters were pH, vitamin C content, Total Soluble Solid (TSS), total phenolic content, total flavonoid content and antioxidant activity by DPPH scavenging method. Each of mentioned parameters of tea were observed for 17 days, with 2 days interval measurement. The treatment was replicated five times. The results in averaged time showed that liang tea with pH 4 possess highest total phenolic content and TSS (13.04 ± 1.21 mgGAE/g dw and 2.40 ± 0.3°Brix respectively) and pH 5 liang tea possess highest vitamin C, total flavonoid content and antioxidant activity (5.49 ± 0.94 mg/100ml; 20.02 ± 2.12 mgQE/g dw and 40.38 ± 4.37 % respectively. Base on the result showed the optimum pH of liang tea during storage was ranged 4 to 5, which pH 4 liang tea possess highest total phenolic content and total dissolved solid and pH 5 liang tea possess highest vitamin C, total flavonoid content and antioxidant activity.*

***Keywords: antioxidant; liang tea; pH; phytochemical; storage time***

## INTRODUCTION

Tea is the most consumed beverage in the world wide beside water and highly appreciated for its attractive taste and aroma,health benefit and socio-cultural characteristic (Oduro et al., 2019; Kinki, 2021). Tea defined as a plant parts made product (whole, fragmented or ground) obtained by appropriate process and used in food beverages by infusion or decoction in water and may not serve for pharmacotherapeutic purpose (Rotta et al., 2016). Tea usually refers to herbal teas/tisanes produced from water infusion of plant species other than *Camellia sinensis* that consist of fresh or dried plant materials such as leaves, fruits, flowers, barks, roots from single or multiple species (Liu et al., 2013).

Tea has various beneficial health effect due to its high content of polyphenols from plant materials (Rotta et al., 2016). Despite a lot of potential health benefit that has been revealed, it is important to understand the stability of tea polyphenol in foods or drinks during processing and storage in order to gain the optimum health benefits from them (Zeng et al., 2017). Xu et al. (2019) reported that several factor, which pH is the critical factor could affect the stability of epigallocatechin-3-gallate (EGCG), one of the polyphenol compounds in tea. According to Tewari et al. (2000), lemon juice addition in green tea could enhanced antioxidant and pharmacological activities, which explain the effect of ascorbic acid on the increase of stability of polyphenols due to lowered pH. Based on this fact, the study about pH conditioning of herbal tea is important and need to be explored in order to maintain the polyphenolic content of tea and optimize their health properties effect. The purpose of this research is to find out the effect of pH on phytochemical and antioxidant of liang tea during storage time.

## METHODOLOGY

### Materials

The leaves of *A.vera, P.amaryllifolius, O.aristatus, R.discolor, D.chinensis* and wood of *C.sappan* were brought from local marketplace in Pontianak City. The chemicals and reagents used were analytical grade. Gallic acid, natrium carbonate ($Na_2CO_3$), *folin-ciocalteu* reagent, sodium phosphate dibasis ($Na_2HPO_4$), citric acid, ethanol, aluminium(III) chloride ($AlCl_3$), $NaNO_2$, quercetin, natrium hydroxide (NaOH), iodine, potassium iodide and starch were obtained from Merck (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and were obtained from Sigma (Sigma-Aldrich,Germany). Aquabidest were obtained from local chemical store.

### Sample Preparation

There are six materials of liang tea that is of *Dicliptera chinensis, Pandanus amaryllifolius, Caesalpinia sappan, Aloevera chinensis, Oreganum vulgare* and *Tradescantia spathacea.* Tea composition were shown in Table 1. The materials were washed, cleaned carefully, and dried using a cabinet dryer at 50$^{o}$C for 12 hours. The dried materials were finely powdered with the grinder and sieved to 80-mesh size. The herbs powder was mixed according to the treatment and the tea was prepared by performed by Taufik *et al.* (2016) with some modification. 1 sachet of liang tea according of the treatment were transfered to beaker glass then heated with 200ml aquabidest at 80-90$^{o}$C and stirred with magnetic rod for 6 minutes. All of teas were added xylitol as sweetener. The tea were stored in refrigerator with 4$^{o}$C for 24 hours and filtrated through Whatman paper no.1. The filtrate of liang tea then stored at freezer until further analysis.

Table 1. Herb composition for each formulation in tea sachet

| Ingredients | Formulation (g) |
|---|---|
| *Dicliptera chinensis* | 1 |
| *Pandanus amaryllifolius* | 0.2 |
| *Caesalpinia sappan* | 0.2 |
| *Aloevera chinensis* | 0.2 |
| *Origanum vulgare* | 0.2 |
| *Tradescantia spathacea* | 0.75 |
| Total | 2.55 |

## Parameter

### pH

The pH was measured by using pH meter (AMT20) and standardized by buffer solutions of pH 4, 7 and 10 following the method as described in AOAC (2007).

### Total Soluble Solid

The total soluble solids expressed as degree brix were analyzed using hand refractometer at room temperature following the procedure of Kusumiyati et al. (2020).

**Vitamin C**

Vitamin C determination were carried out by iodine titration following the procedure of Alija et al. (2017)

**Determination of Total Phenolic Content (TPC)**

Total phenolic content of tea was determined according to the method based on Quan et al. (2014) with some modification. Tea sample (0.5 ml) followed by Folin-Ciocalteu reagent (2.5ml) was added to testing tube and let it stand for 5 minutes at dark and ambient temperature. After that, 7.5% (w/v) $Na_2CO_3$ was added to the solution and the mixture were homogenized using vortex mixer and placed in dark place at room temperature. A blank solution was prepared with the same manner by using dd$H_2O$ (0.5ml). Absorbance were measured at 765 nm using spectrophotometer. The results were expressed in gallic acid equivalents per gram of fresh weight of pineapple peel through gallic acid calibration curve (0 – 100 µg/mL).

**Determination of Total Flavonoid Content (TFC)**

Total flavonoid content by aluminium chloride method was performed based on Jaiswal et al. (2012). Each tea sample (250 µL) followed by dd$H_2O$ (1.25ml) and lastly 5% sodium nitrite solution (75 µL) were added to testing tube. After 6 minutes, 10% aluminium chloride solution (150 µL) was added along with 1M sodium hydroxide (0.5ml) and dd$H_2O$ (575 µL) was added to bring the total volume to 2.5 ml. A blank solution was prepared in the same procedure by using dd$H_2O$ instead of tea sample. Absorbance were measured at 510 nm. The results were expressed in quercetin equivalents per gram of fresh weight of pineapple peel through quercetin calibration curve (0 – 140 µg/mL).

**Determination of Antioxidant Activity**

The determination of antioxidant activity by DPPH scavenging method was performed according to a method described on Nguyen and Chuyen (2020) with some modification. The DPPH solution was prepared by diluting stock DPPH solution (0.24g/L) with methanol until the absorbance reached 1.1 ± 0.01 at 515nm. Tea (0.15ml) was mixed with DPPH solution (2.85ml) and the mixture was left for 15 minutes in dark place at room temperature. The solution then measured using spectrophotometer at 515 nm wavelength. A blank solution also prepared with the same procedure by using methanol instead of tea sample (0,15ml). Antioxidant by DPPH scavenging activity was calculated using the following formula :

$$\text{DPPH inhibition (\%)} = [(A_b - A)/\ A_b] \times 100$$

Where $A_b$= absorbance of control (blank) and $A_e$ = absorbance of tea sample.

**Statistical Analysis**

This study using Randomized Group Design with 2 factors that is pH with 4 treatments (4, 5, 6, 6,5) and storage time (day 1,3,5,7,9,11,13,15,17). The data was analyzed as mean ± standard deviation values by repeating the procedures in five replications. The standard deviation values were determined by using one-way analysis of variance (ANOVA). The statistical data was analysed by using Microsoft Excel and visualised using charts.

## RESULTS AND DISCUSSION

### Effect of Storage Time on pH

The effect of storage time of different initial pH of liang tea on pH change data were shown in figure 1. The highest pH on day 1 obtained from pH 6.5 treatment (5.28 ± 0.07) and the lowest pH obtained from pH 4 treatment (4.26 ± 0.01) and gradually decreased until the last day of experiment, which is the highest was on pH 5 treatment (4.20 ± 0.34) and the lowest obtained from pH 4 treatment (3.75 ± 0.21). Both pH, storage time and their interaction showed significantly different on pH value statistically. The averaged pH value of storage time ranged from 3.95 ± 0.23 to 4.55 ± 0.46, in order from the lowest value which is pH 4, 6.5, 6 and 5 respectively. The value of pH decrease during fermentation process can encourage the hydrolysis process which caused increase in glucose and fructose levels(Kang, 2016). In another word, the decrease of pH is closely related to the increase of brix value (Anoraga, 2021).

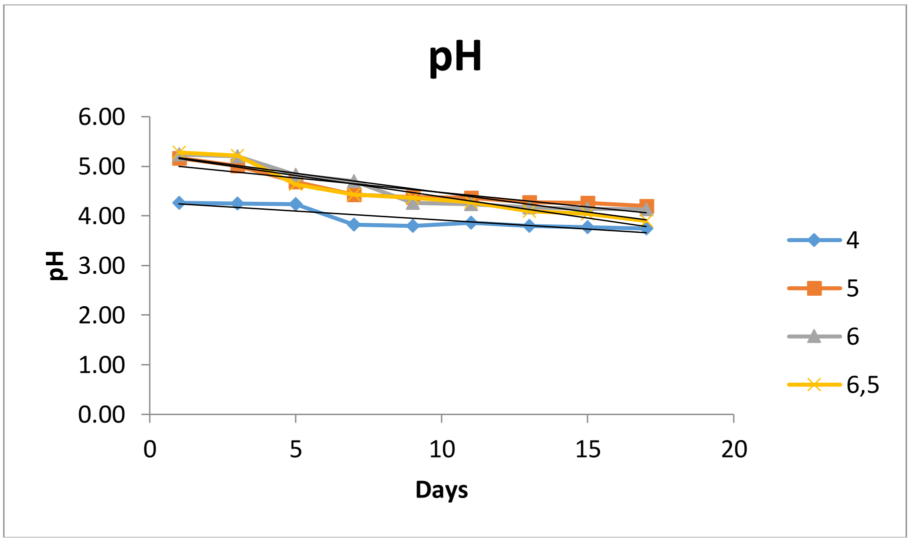


Figure 1. Trend pH of liang tea during storage

Table 2. Description figure 1

| Treatment | Regression Equation | $R^2$* | $\bar{\Delta}$ pH** |
|---|---|---|---|
| pH 6.5 | y=-0.036x+4.279 | 0.762 | 0.91 ± 0.48 |
| pH 6 | y=-0.058x+5.057 | 0.846 | 0.78 ± 0.17 |
| pH 5 | y=-0.078x+5.250 | 0.879 | 0.71 ± 0.10 |
| pH 4 | y=-0.085x+5.241 | 0.911 | 0.35 ± 0.01 |

*$R^2$ = Coefficient of determination
**$\bar{\Delta}$ = Difference of values between first day until last observed storage time

### Total Soluble Solid

The effect of storage time of different initial pH of liang tea on total dissolved solid were displayed on table 2. The data showed fluctually from each examined day along the storage time and based on averaged value, total dissolved solid of liang tea ranged from 2.16 ± 0.18 to 2.40 ± 0.30°Brix, in order from the lowest value that is pH 6.5, 6, 5, and 4 respectively. Total dissolved solids increased which is influenced by polysaccharide hydrolysis and the increase of carbohydrates due to the enzymatic heating process that convert carbohydrates into sugars (Nelwida et al., 2019). The longer the heating, more soluble the sugars that cause total dissolved solids increase (Anoraga, 2021). According to Meena et al. (2017), retention or minimum increase in TSS content of juice during storage is desirable for preservation of good juice quality.

**Vitamin C Content**

The effect of storage time of different initial pH of liang tea on Vitamin C content were shown in figure 3. The data showed vitamin C content gradually decreased from day 1 until the last day. Based on averaged value of each storage time, vitamin C content ranged from 4.24 ± 0.62 to 5.49 ± 0.94 mg/100ml, in order from the lowest value that is pH 4, 6, 6.5 and 5 respectively. Similar result is reported by Kilima et al. (2014) which observing influence of storage time and temperature on ascorbic acid of roselle-fruit juice from 0 to 6 months. The loss of organic acids on beverages during storage mainly affected by anaerobic degradation and it is influenced by temperature, light and storage time (Randhawa et al., 2014). Vitamin C or ascorbic acid is reversibly oxidized into dehydroascorbic acid (DHA) upon exposure of light, heat, transition metal ions and pH (alkaline condition)(Yin et al., 2022).

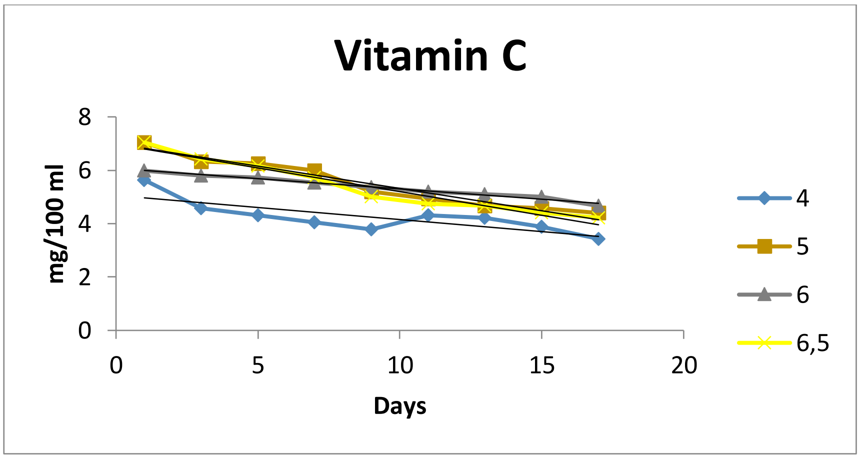


Figure 2. Trend vitamin C of liang tea during storage

Table 3. Description figure 2

| Treatment | Regression Equation | $R^2$* | $\bar{\Delta}$ Vitamin C (mg /100ml)** |
|---|---|---|---|
| pH 6.5 | Y=-0.177x+6.975 | 0.956 | 1.87 ± 0.51 |
| pH 6 | y=-0.077x+6.070 | 0.983 | 0.68 ± 0.48 |
| pH 5 | y=-0.167x+6.990 | 0.952 | 1.75 ± 0.47 |
| pH 4 | y=-0.090x+5.055 | 0.631 | 1.56 ± 0.33 |

*$R^2$ = Coefficient of determination
**$\bar{\Delta}$ = Difference of values between first day until last observed storage time

**Total Phenolic Content**

The effect of storage time of different initial pH of liang tea on total phenolic content were displayed on figure 4. Total phenolic content data of liang tea changed fluctually from each observed day. Both pH, storage time and their interaction showed significantly different on total phenolic content of liang tea statistically. Based on averaged value of storage time, total phenolic content of liang tea ranged from 10.65 ± 1.35 to 13.04 ± 1.21 mg GAE/g fw, in order from the highest value that is pH 4, 5, 6, and 6.5 respectively. The result of this study is similar to Chang et al. (2020) which study on infusion effect and storage time on black tea. Tea polyphenol was known to be degraded faster with the increase of either pH, oxygen concentration, or temperature (Zimeri & Tong, 1999). The main factor that affect the

Table 4. Trend total soluble solid of liang tea during storage

| Treatment | Observed Day (°Brix) | | | | | | | | | Regression Equation | $R^2$* | $\bar{\Delta}$ TSS (°Brix)** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 3 | 5 | 7 | 9 | 11 | 13 | 15 | 17 | | | |
| pH 6.5 | 2.28±0.11 | 2±0.00 | 2.12±0.11 | 2.4±0.00 | 2.2± 0.14 | 2.16±0.09 | 2.4±0.00 | 1.92±0.11 | 1.96 ±0.09 | -0.01x+2.25 | 0.093 | 0.14±0.12 |
| pH 6 | 2.04±0.09 | 2.2±0.00 | 2.32±0.11 | 2.2±0.00 | 2.24±0.09 | 2.52± 0.11 | 2.56±0.09 | 2.12±0.11 | 2.04 ±0.09 | 0.004x+2.206 | 0.018 | 0.23±0.09 |
| pH 5 | 2.82±0.04 | 2.24±0.09 | 2.64±0.09 | 2.36±0.09 | 2.2±0.00 | 2.24±0.09 | 2.8±0.00 | 2.12±0.11 | 2±0.00 | -0.028x+2.638 | 0.273 | 0.5±0.04 |
| pH 4 | 2.92±0.11 | 2.12±0.11 | 2.12±0.11 | 2.2±0.00 | 2.24±0.09 | 2.32±0.11 | 2.88±0.11 | 2.36±0.09 | 2.44±0.17 | 0.003x+2.367 | 0.004 | 0.58±0.14 |

*$R^2$ = Coefficient of determination

**$\bar{\Delta}$ = Difference of values between first day until last observed storage time stability of polyphenols in fruits and vegetables is the pH and generally polyphenols more stable as the pH value of the solution is lower. The variation of pH value could influence the stability of polyphenols by changing their chemical structure, which is also responsible for color variation of polyphenols (Cao et al., 2021). Yu et al. (2022) reported that aqueous polyphenol content was stable under the condition of pH 3.0 – 5.0. Another study reported that storage conditions can affect the polyphenol content mainly due to hydrolysis, oxidations and complications with storage at higher temperatures (Zafrilla et al., 2003).

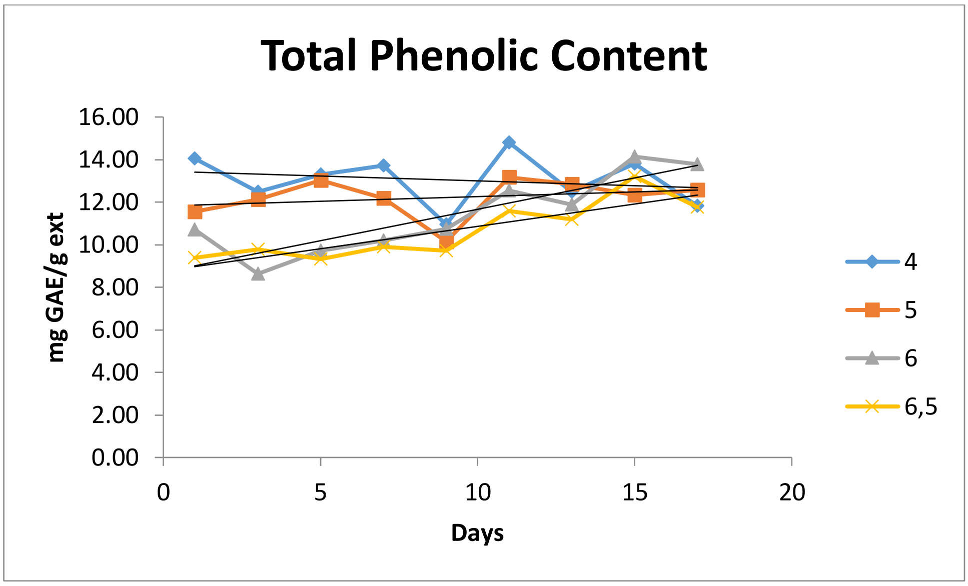


Figure 3. trend total phenolic content of liang tea during storage

Table 5. Description figure 3

| Treatment | Regression Equation | $R^2$* | $\bar{\Delta}$ TPC (mg GAE/ml ext)** |
|---|---|---|---|
| pH 6.5 | -0.045x+13.45 | 0.73 | 1.43 ± 1.09 |
| pH 6 | 0.044x+11.81 | 0.761 | 0.75 ± 0.33 |
| pH 5 | 0.295x+8.709 | 0.168 | 0.74 ± 0.32 |
| pH 4 | 0.210x+8.756 | 0.042 | 1.13 ± 0.42 |

*$R^2$ = Coefficient of determination
**$\bar{\Delta}$ = Difference of values between first day until last observed storage time

**Total Flavonoid Content**

The effect of storage time of different initial pH of liang tea on flavonoid content were shown in figure 5. Total flavonoid content of liang tea changed fluctually from each observed day. Both pH, storage time and their interaction showed significantly different on total flavonoid content statistically. Based on averaged value of storage time, total flavonoid content ranged from 17.04 ± 3.72 to 20.02 ± 2.12 mg QE/g fw, in order from the highest value that is pH 5, 6, 6.5 and 4 respectively. This results similar to Wurzbach et al. (2015) that study about storage duration on stability of hops and green tea extract from 0 to 13 months. pH value has important influence on the degradation of flavonoids. Flavonoids show instability in aqueous solution and susceptible to degradation and the main degradation reaction involve hydroxylation, oxidation, and ring-cleavage (Wang & Zhao, 2016).

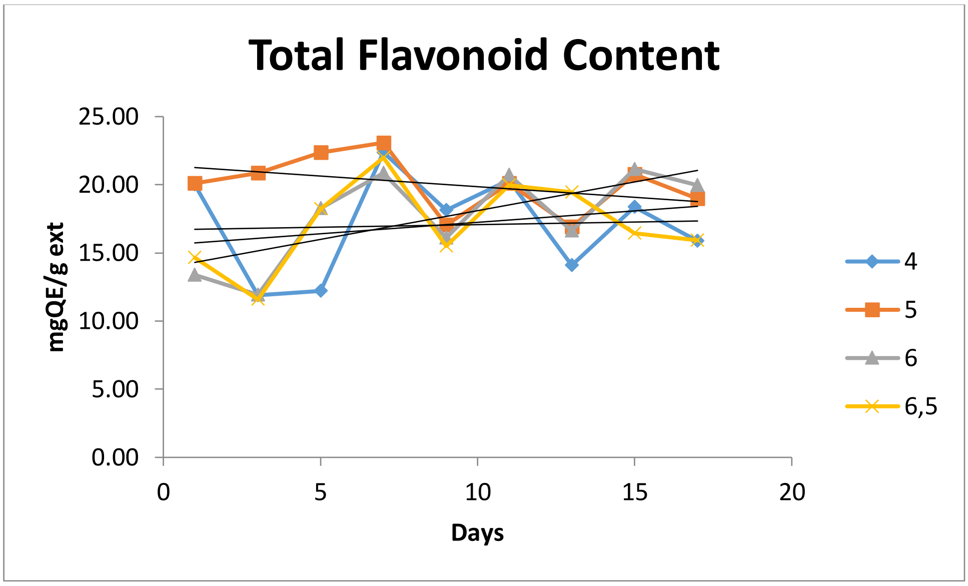


Figure 4. Trend Total Flavonoid Content of Liang Tea During Storage

Table 6. Description figure 4

| Treatment | Regression Equation | $R^2$* | $\bar{\Delta}$ TFC (mgQE/ml ext)** |
|---|---|---|---|
| pH 6.5 | 0.167x+15.56 | 0.084 | 2.74 ± 1.72 |
| pH 6 | 0.421x+13.88 | 0.464 | 4.80 ± 2.38 |
| pH 5 | -0.156x+21.42 | 0.162 | 0.09 ± 3.78 |
| pH 4 | 0.038x+16.69 | 0.003 | 3.36 ± 4.17 |

*$R^2$ = Coefficient of determination
**$\bar{\Delta}$ = Difference of values between first day until last observed storage time

**Antioxidant Activity by DPPH Scavenging Method**

The effect of storage time of different initial pH of liang tea on antioxidant activity were shown in figure 6. Antioxidant activity of liang tea changed fluctually for several observed day until the last storage time. Both pH, storage time and their interaction showed significantly different on antioxidant activity of liang tea statistically. Based on averaged value of storage time, antioxidant activity of liang tea ranged from 36.64 ± 8.62 to 40.38 ± 4.37, in order from the highest value that is pH 5, 4, 6 and 6.5 respectively. The result show similarities with Chang et al. (2020) which study on infusion effect and storage time on black tea. Several study reported that plant phenols exhibit prooxidant and cytotoxic properties in certain conditions. The presence of oxygen or transitional metal ions and alkaline pH conditions triggers prooxidant activity of phytochemical (Bayliak et al., 2016).

Table 7. Description figure 6

| Treatment | Regresion Equation | $R^2$* | $\bar{\Delta}$ antioxidant (%)** |
|---|---|---|---|
| pH 6.5 | 1.072x+26.99 | 0.463 | 7.06 ± 0.84 |
| pH 6 | 1.354x+24.82 | 0.608 | 4.23 ± 2.50 |
| pH 5 | 0.614x+34.84 | 0.593 | 3.71 ± 4.99 |
| pH 4 | 0.369x+34.16 | 0.139 | 1.90 ± 1.28 |

*$R^2$ = Coefficient of determination
**$\bar{\Delta}$ = Difference of values between first day until last observed storage time

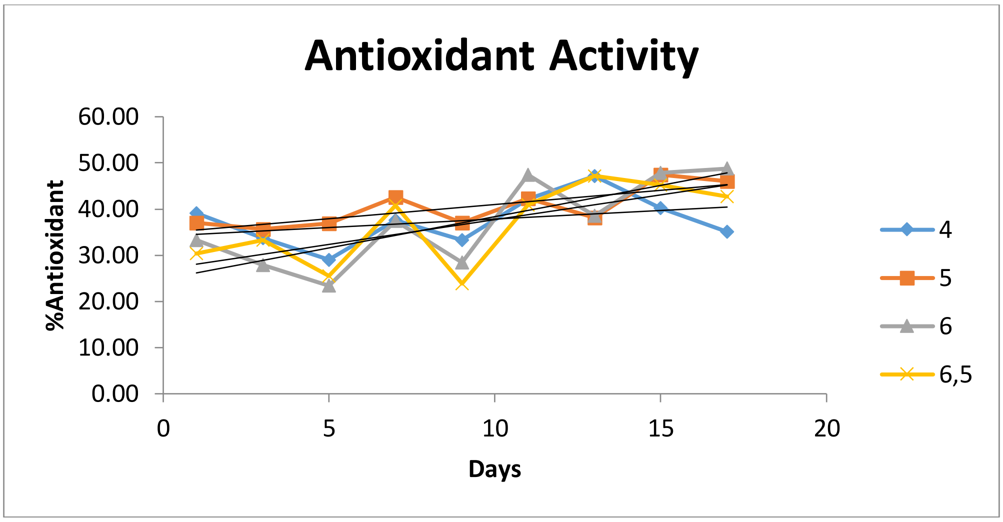


Figure 6. Trend antioxidant activity of liang tea during storage

## CONCLUSIONS

The optimum pH of liang tea during storage was ranged 4 to 5, which pH 4 liang tea possess highest total phenolic content and total dissolved solid and pH 5 liang tea possess highest vitamin C, total flavonoid content and antioxidant activity.

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