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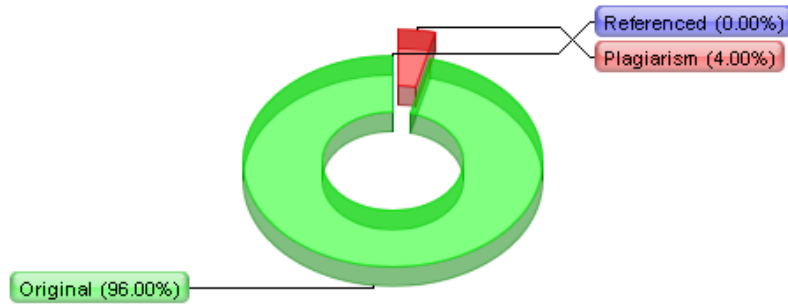
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phenolic content and antioxidant activity of

fig fruit juice. Fig fruit juice was fermented at 37°C for 24 hours using 4 types of starter bacteria as *Lactobacillus acidophilus*, *Lb. bulgaricus*, *Lb. casei* and *Lb. plantarum*. The total phenolic content testing was conducted using the spectrophotometry method using sulfanilic acid reagent.

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The antioxidant activity was tested using the

spectrophotometry method using DPPH reagent. The fermentation result of fig fruit juice using *Lactobacillus bulgaricus* demonstrates the highest increase of total phenolic content (TPC=0.45%) and the biggest antioxidant activity (IC50=76.55 ppm) compared to the unfermented ones (TPC=0.09%; IC50=76.7 ppm). The fermentation result of fig fruit juice using other bacteria also demonstrates the increase of total phenolic content with a smaller antioxidant activity compared to the unfermented ones, yet it is still included as a strong activity. Based on the results,

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it can be concluded that the

lactic acid fermentation is able to increase the total phenolic content and preserve the antioxidant activity. Keywords: antioxidant, fig, total phenolic content. INTRODUCTION

Fig fruit is commonly known by the society as a fruit having which has many benefits for the health. It is mostly used as an anti-cancer, degenerative disease therapy, digestion problem, osteoporosis and infectious disease prevention due to its quite complete nutrition contents. So, this fruit is known as a paradise fruit.

Some researchers have demonstrated various fig fruit contents to support its use for the society. Joseph and Raj (2011) in Soni et al. (2014) stated that different parts of plant as fruit, seed, leaf, stem, bud, and sap have various benefits for the health. Dried fig fruit has been known as a source of carbohydrate, sugar, mineral, vitamin, organic acid, and phenolic compounds (Veberic et al., 2008; Jeong and Lachance, 2001; Slatnar et al., 2011 in Soni et al., 2014). Fig

fruit contains rather high phenolic compounds to support its use as an antioxidant. Yet, most of the phenolic compounds are difficult to be absorbed by the human body. Manach et al., 2014 in Filannino et al., (2016) stated that the highest phenolic compounds are found in the vegetables, except for flavonol which is found in the form of glycosylated and glycosylation affecting the absorption. To ease the absorption, the hydrolysis by enzymes or microbes is required in the digestive tract. In line with the previous statement, the fig fruit contents require the hydrolysis processes, one of them is fermentation process. Fermentation can

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increase the nutrition value in the

foodstuffs. It can also increase the antioxidant activity in the fermented materials. Zhang et al. (2012) stated that fermentation can increase the antioxidative activity by increasing the flavonoid release of plant-based food.

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biochemical changes occurred during the fermentation

causing the nutrition component and anti-nutrition ratio changes influencing the product characteristics, as a bioactivity and digestibility. According to Hur et al. (2014), the glycoside conversion into the aglycone by the fermentation is the main principle of the antioxidative activity increase in the plant-based food. The most applied fermentation in the foodstuffs is a lactic acid fermentation. This fermentation type uses lactic acid bacteria which are proven their safety and benefits. *Lactobacillus plantarum* is the most used species for the fermentation in the plant-based food products. Other lactic acid bacteria are *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus fermentum*, *Bifidobacterium animalis* subsp. *lactis* and *Bifidobacterium longum* (Rodríguez et al., 2009; Marazza et al., 2009). Therefore, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei* and *Lactobacillus plantarum* are used as a starter in this research. This research aims to determine the

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phenolic content and antioxidant activity of

fig fruit juice. MATERIAL

S AND METHOD Material

Dried fig fruit was purchased from the tin leaf tea producer (Kunta tea) in Gresik. *Lactobacillus acidophilus* and *Lactobacillus plantarum* bacterial cultures were purchased from the Microbiology Laboratory, Agricultural Product

Technology Department, Agricultural Technology Faculty, Brawijaya University, Malang, while *Lactobacillus bulgaricus* and *Lactobacillus casei* cultures were isolated from the commercial fermented products. Fig Fruit Juice Fermentation

Dried fig fruit was blended and added with water with the ratio of 1:5. Then, the obtained juice was filtered to gain the fig fruit juice. The fig fruit juice was pasteurized for 15 minutes at 72

°C, then was left until the temperature dropped into around 40°C. After that, the juice was added with the bacterial starter (6%) and incubated at 37°C for 24 hours. Total Phenolic Content (TPC)

Testing Some samples were put into the test tube, added with 1 ml of reagent A (Sulfanilic acid 7.64%, H₂SO₄, and Sodium Nitrite 4.8 %) and 0.5 ml NaOH 8%, then shaken and left for 3 minutes to form a color. Then, it was measured for its absorbance using spectrophotometer at 360 nm of wave length. The standard curve was made of phenol with the same procedure. Antioxidant Activity Testing

Some samples were weighed, dissolved in methanol

, added with an absorbent to omit the unwanted compounds. The absorbent contained the mix of Al, Mg, SO₄, and Si added with sodium acetate as its buffer. Then, the samples were centrifuged for 10 minutes at 4500 rpm of speed, added with methanol and DPPH, incubated in the dark room, then measured its absorbance. The quercetin standard was made using several concentrations (0.5 ppm; 1 ppm; 2 ppm; 4 ppm and 8 ppm), added with methanol and DPPH, incubated in the dark room, measured for its absorbance using spectrophotometer at 517 nm of wave length. RESULTS

AND DISCUSSION Effect of Lactic Acid Fermentation on Phenolic Compounds

All of the lactic acid bacteria used as starters in this research is able to ferment the fig fruit juice and produce the increase of total phenolic content significantly. Before fermented, the total phenolic content of fig fruit juice were 0.09%, while after fermented, the total phenolic content increase between 100% to 400%. The highest increase was obtained from the fermentation result using *Lactobacillus bulgaricus* producing the total phenolic content of 0.45%. The fermentation using *L. plantarum* producing the total phenolic content of 0.33%, using *L. acidophilus* producing the total phenolic content of 0.21%, and using *L. casei* producing the total phenolic content of 0.19% (Figure 1). Figure

1. Total Phenolic Content of Fig Fruit Juices. NF=Non Fermented; FlA= Fermented with *Lactobacillus acidophilus*; FLb= Fermented with *Lactobacillus bulgaricus*; FLC= Fermented with *Lactobacillus casei*; FLp= Fermented with *Lactobacillus plantarum*. The different alphabetical notations show the significant differences based on the Tukey HSD testing with the confidence interval of 95%. The phenolic compounds were known as a secondary metabolite, which is important, coming from the phenylalanine and tyrosine. These compounds contain in a large amount variously in plants (Kahkonen et al., 1999 in Nithya et al., 2016). Plant phenolic compounds are important because their hydroxyl group can prevent the free radicals. Plant materials containing phenolic compounds are increasingly used in the food industries because they can prevent the oxidative degradation in fat and increase

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the quality and nutritional value of food (Naczka and Shahidi, 2004

in Nithya et al., 2016). Based on the total phenolic content determination result of fig fruit juice, the increase of total phenolic content after the juice experiencing the lactic acid fermentation can be determined. It shows that the lactic acid fermentation can increase the total phenolic content of fig fruit juice. The increase of total phenolic content can occur due to the lactic acid bacteria activity used as starters in the fermentation process. According to Messen and Vuyst (2002) in Nazarni et al. (2016), the fermentation process causes the microbial enzyme release producing higher chemical compounds from the plant as flavonoid, tannin, alkaloid, and phenylpropanoid. Othman et al. (2009) stated that the lactic acid bacteria existence in the fermentation contributes to the simple phenolic conversion and phenolic compounds depolymerization with the high molecular weight. Duenas

et al. (2005) stated that the increase of total phenolic content in the fermentation result is caused by the enzymatical reaction in the substrate, so it releases rather high phenolic compounds as a final product. The natural fermentation using microorganisms stimulates the pH reduction so that several involved enzymes in the complex polyphenols hydrolysis were activated resulting on the active, simpler and higher polyphenols. During the fermentation, the β -glucosidase from the microbes can hydrolyze phenolic and flavonoid. *L. plantarum* was reported to have a strong glucosidase activity. Thus, the active substance experiencing increase is expected to be converted from the enzymatic cleavage of corresponding glucosides. Zhang et al. (2012) also stated that the fermentation can induce the cell wall structural break down causing the bioactive compounds release and/or synthesis. During the fermentation, the bound phenol can be released enzymatically. The increase

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of total phenolic compounds in the

fermentation of fig fruit juice is in accordance with some of the reported studies as the increase of total phenolic content on *Graptopetalum paraguayense* E. Walther (Wu et al., 2011), *Echinacea* spp. (Rizello, 2013), Malaysian herbal teas (Ibrahim et al., 2014) and jaruk tigarun flowers (Nazarni et al., 2016). Ng et al. (2011) also reported that parts of plants experiencing the increase of total phenolic content after the fermentation. Effect of Lactic Acid Fermentation on Antioxidant Activity

The lactic acid fermentation on the fig fruit juice produces a various antioxidant activity. The antioxidant activity was demonstrated by the IC₅₀ value. IC₅₀ or inhibition concentration 50 is an antioxidant concentration required

to inhibit 50% of free radicals. The higher of IC50 value means the lower of antioxidant activity. Mishra et al. (2012) stated that IC50 is defined as the antioxidant amount required for reducing 50% of DPPH absorbance from the early absorbance. Before fermented, the fig fruit juice showed 76.7 ppm of IC50 value. The fermentation using *L. bulgaricus* shows the increase of antioxidant activity with the IC50 value of 76.55 ppm, yet the increase is not significant. The fermentation using *L. casei* shows the reduction of antioxidant activity with the IC50 value of 77.41 ppm, yet the reduction is not significant too. The fermentation using *L. acidophilus* and using *L. plantarum* produce the significant reduction of antioxidant activity with the IC50 value of 105.42 ppm and 95.94 ppm. In conclusion, the lactic acid fermentation can affect the antioxidant activity of fig fruit juice. Figure 2. IC50 of Fig Fruit Juice. NF=Non Fermented; Fla= Fermented with *Lactobacillus acidophilus*; FLb= Fermented with *Lactobacillus bulgaricus*; FLc= Fermented with *Lactobacillus casei*; FLp= Fermented with *Lactobacillus plantarum*. The different alphabetical notations show the significant differences based on the Tukey HSD testing with the confidence interval of 95%. If observed from its IC50 value, the fermentation using *L. bulgaricus*, *L. casei*, and *L. plantarum* produces the active antioxidant activity, whereas the fermentation using *L. acidophilus* produces the average antioxidant activity. It refers to Jun et al. (2003) in Salusu et al. (2017) stating that the antioxidant activity power is determined by the IC50 value, where less than 50 ppm classified as strong, 50-100 ppm classified as active, 100-250 ppm classified as average, 250-500 ppm classified as weak and more than 500 ppm classified as inactive. The antioxidant activity change after the fermentation process can be affected by the starter bacteria used. Zhang et al. (2012) stated that the fermentation can affect the total phenolic content and antioxidative activity with the level of influence depending on the species of microorganisms used.

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The fermentation process is supposed to increase the antioxidant activity. Marazza et al. (2009) stated that the lactic acid bacteria with the β -glucosidase activity (including *Lactobacillus acidophilus*, *L. casei*, *L. plantarum*, *L. fermentum*, *Bifidobacterium animalis* subsp. *lactis* and *Bifidobacterium longum*) can increase the aglycone during the fermentation. The aglycone acts as an antioxidant. The finding shows that *L. bulgaricus* and *L. casei* can produce the fermented fig fruit juice by retaining the antioxidant activity demonstrated by the insignificantly different IC50 value. Nevertheless, *L. acidophilus* and *L. plantarum* produce the fermented fig fruit juice with a lower antioxidant activity compared to the activity before the fermentation. The exact same thing also happens in the Chetoui olives (*Olea europaea* L.) fermentation with the reduction of antioxidant activity approximately between 50% and 72% (Othman et al., 2009). The antioxidant activity of fig fruits juice can be produced by the secondary metabolite compounds in fig fruit. Soni et al. (2014) stated that in the dried fig fruit there are phenolic (10.90 μg GAE/mg sample), flavonoid (2.75 μg CE/ mg sample), alkaloid (9.6%) and saponin (0.59%). Aksov et al. (2013) stated that there is an antioxidant compound in the form of phenolic. The antioxidant characteristics of phenolic compounds come from the proton-releasing ability, chelate formation and radical dismutation. The phenolic compounds give the hydrogen atom from hydroxyl group to the radical and form a stable phenoxyl radical so it gives a significant role in the antioxidant activity. Therefore, the determination of phenolic total content is very important to determine the antioxidant ability of plant extracts. The increase of phenolic compounds is supposed to produce a higher antioxidant activity since there is more hydroxyl group capable of preventing the free radicals. Yet, in this research,

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the antioxidant activity produced by the fermented fig fruit juice does not experience any increase as occurred in the total phenolic content. It shows that the hydroxyl group number of phenolic compounds is not the only factor determining the antioxidant activity. Some studies state that the antioxidant activity structure of flavonoid and phenolic acid depending on the hydroxyl group position of phenolic and the existence of other functional group on the whole molecule as double bond and its conjugation towards the hydroxyl and ketone groups (Rice-Evans & Miller, 1996; Silva et al., 2002 in Mishra et al., 2012). Several studies indeed show the positive correlation between the antioxidant activity and total phenolic content, as in the antioxidant activity of buckwheat (*Fagopyrum esculentum* Moench) (Sun and Ho, 2005), ulam raja' (*Cosmos caudatus*), 'kesum' (*Polygonum minus*), 'selom' (*Oenanthe javanica*), 'pegaga' (*Centella asiatica*) and 'curry leaf' (*Murraya koenigii*) (Faujan et al., 2009), *Radix Angelicae Sinensis* (Li et al., 2009), *Solanum tuberosum* (Hesam et al., 2012) and *Thermopsis turcica* (Aksoy et al., 2013). Yet, the other studies demonstrate the contrast result showing the negative correlation between the antioxidant activity and total phenolic content, as in the antioxidant potency of *Chenopodium quinoa* and *Amaranthus* spp seeds obtained by using 3 different methods (FRAP, DPPH and carotene bleaching) that negatively correlates with the total phenolic content (Nsimba et al., 2008). The negative correlation was also found in the antioxidant activity and total phenolic content of 15 genotypes of selected *Zizyphus jujube* Mill. from Turkey (Kamiloglu et al., 2009). Also, Rafat et al. (2010) demonstrated the negative correlation between the antioxidant activity and total phenolic content of ethanolic extract of selected plants. The ethanolic extract of *Euodia redlevi* contains the highest phenolic compounds compared to the phenolic compounds of other extracts. The lowest total phenolic content were demonstrated by the extract of *Centella asiatica*, yet it produces the second highest antioxidant activity, both in the DPPH and SOD methods. Based on the previous statements, there is a possibility causing the reduction of antioxidant activity of fermented fig fruit juice although there is an increase of total phenolic content. First, there is

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a change in the structure of

hydroxyl group of phenolic compounds of fig fruit after the fermentation process, so it does not produce the increase of antioxidant activity even when there is an increase of total phenolic content. Another possibility is the non-phenolic compound existence of fig fruit having a role in the antioxidant activity ability, CONCLUSION

T

he lactic acid fermentation is able to increase the total phenolic content significantly and preserve the antioxidant activity, which is classified as a strong category. The increase of total phenolic content of fig fruit juice after the fermentation is not followed by the antioxidant activity increase. It is due to the hydroxyl group structure change or the non-phenolic compound existence acting as an antioxidant. ACKNOWLEDGEMENT

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