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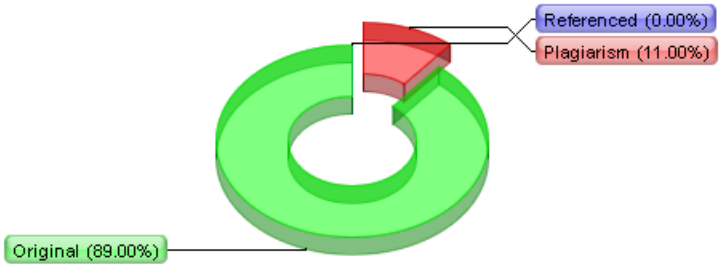

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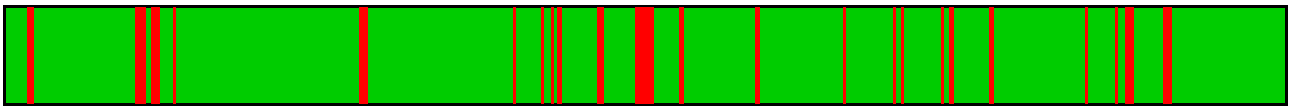

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THE EFFECT OF LACTIC ACID FERMENTATIONON FIG (Ficus carica) FRUIT FLAVONOID Abstract
Fig fruit contains a quite high flavonoid that supports the use of it for several dis
ease therapies. Yet, most of the flavonoid in plants is difficult to be digested since it bounds with the glycoside, so the hydrolysis is necessary. The hydrolysis can be done through

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the lactic acid fermentation. This research aims to determine the effect of lactic acid fermentation on fig fruit flavonoid. Dried fig fruit was prepared into a fig fruit extract and fermented at 37'C for 24 hours using 4 types of starter bacteria; Lactobacillus acidophilus, L. bulgaricus, L. casei and L. plantarum. The fermentation result was identified its flavonoid using dye reaction, determined its flavonoid content using the spectrophotometry with the quercetin standard, and analyzed qualitatively using the LC-MS/MS. The results show that the flavonoid was identified in both before and after the fermentation, but the flavonoid content decreases 30 - 50% after the fermentation. The LC-MS/MS shows that the identified flavonoid is rutin, with the relatively higher percentage after the fermentation. In addition, the catechin and epicatechin are not detected. It can be concluded that the lactic acid fermentation affects the fig fruit flavonoid. The fermentation with all types of starter bacteria decreases the total flavonoid content of fig fruit juice.Key words: flavonoid, fig, lactic acid fermentation

INTRODUCTION

Fig fruit is one of the fruits me
ntioned in the Islamic bible (Al-Qur'an). Nowadays, the fig fruit has been mostly cultured in Indonesia because the society already understands its benefits for the health. Some benefits of fig fruit are to prevent cancer, treat degenerative diseases, overcome digestive problems, prevent osteoporosis and overcome infectious diseases.Fig (
Ficus carica) is traditionally used for the health as to repair the metabolism system, cardiovascular, respiratory, and as an antispasmodic and anti-inflammation. The fig

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fruit can be consumed in the fresh or dried condition or made as a jam. The fig fruit juice mixed with honey is used for hemorrhoid. In the Indian medication, the fig fruit is

used as a lactase, expectorant, and diuretic. The fig fruit

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can be also used as the first treatment for liver and lymph diseases. The dried fig fruit can be used as a supplement for the diabetic patients.

The fig fruit pasta can be also used on the tumor swelling and inflammation (Mawa et al., 2013).Some efficacies of fig fruit are supported by its active contents, such as flavonoid.

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The total flavonoid content of the

fig fruit extract was found in a large amount of 2.75 µg CE/ mg of sample (Soni et al., 2014). The fig fruit phenolic acid and flavonoid have been studied, whereas the gallic acid, chlorogenate acid, siringat acid, catechin, epicatechin, and rutin have been identified (Veberic et al, 2008).Flavonoid is an essential compound due to its extensive biological activity, especially as an anti-microbe. It is categorized as the polyphenolic compound because it has ring A and C benzo-1-pyran-4-quinone and ring B (Oskoueian et al., 2013). It is a natural secondary metabolite in the plants that give positive effects to the health. The study of flavonoid derivative shows the flavonoid activity as anti-bacteria, antivirus, anti-inflammation, anticancer, and anti-allergy. The flavonoid demonstrates its ability as a very effective anti-free radical on the oxidative molecules like singlet oxygen and various free radicals causing diseases (Bravo, 1998 in Nithya et al., 2016). It contributes to the induced cell proliferation, apoptosis induction, and enzyme inhibition as well as antibacterial and antioxidant effects (Soni et al., 2014). It has the pharmacology and biochemical effects by inhibiting several enzymes as aldose reductase, Ca2+-ATPase, xanthine oxidase, phosphodiesterase and lipoxygenase. It is also capable of managing some hormones as androgen, estrogen and thyroid (Agrawal, 2011). It has been reported that the bioflavonoid has the protective effect from the DNA destruction induced by the hydroxyl radical. One of the mechanisms explaining the protective effect involves chelating ion of metals as copper or iron. The bioflavonoid forms complexes with copper or iron preventing the ROS formation (Zhou et al., 2001; Rubens & Giovani, 2004; Armida et al., 2005; in Nimse & Pal, 2015).The flavonoid is found in the form of aglycone, glycoside and methylated derivate (Kumar & Pandey, 2013). In plants, it usually exists in the form of glycoside with aglycone bounded in various part of the sugar with the -glycoside binding, especially on the third position of ring C (Oskoueian et al., 2013; Lee et al., 2015). This flavonoid form binding with sugar is difficult to be digested by the human body, so the hydrolysis process is necessary to remove the sugar.Filannino
et al. (2016) stated that the glycosylated flavonoid original form cannot be absorbed by the human body, so it first requires the hydrolysis by the digestive enzyme or intestinal microbiota. In contrast, the aglycone can be directly absorbed by the small intestine. Oskoueian et al. (2013) stated that the part of sugar decreases the flavonoid bioactivity, so removing the sugar part not only increases the functional characteristic but also increases the bioavailability in the digestive tract.One of the methods to remove the sugar part from the flavonoid is by fermentation. The

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fermentation can increase the nutraceutical value of a product by deconstructing certain undesirable compound and inducing the effective microbe (
Oboh et al., 2008). T

he most applied fermentation on a food product is a lactic acid fermentation. The lactic acid bacteria increase the functionality of various vegetable through the enzyme that can stimulate the synthesis of various metabolites or secrete the biogenic compound that does not really appear in the raw material (Gobbetti et al., 2010; Di Cagno et al., 2013 in Filannino et al., 2016).In this research, the fermentation using lactic acid bacteria , which mostly used in the probiotic beverage production as Lactobacillus acidophillus, L. bulgaricus, L. casei and L. plantarum, was conducted. This research aims to determine the effect of lactic acid fermentation on fig fruit flavonoid.METHOD
SMaterials
Dried fig fruit was purchased from the tin leaf tea producer (
Kunta tea) in Gresik. Lactobacillus acidophillus 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.
Flavonoid Identification
1 ml
of sample was added to the evaporating dish, then 3 ml of ethanol 70% was added and shaken. Next, it was heated and shaken again, then filtered. The filtrate was added with 0.1 grams of Mg powder and 2 drops of concentrated HCl. The flavonoid existence was indicated by the red color.Total
Flavonoid Content DeterminationQuercetin Standard Preparation
Quercetin standard was prepared by some concentrations (0.5 ppm, 1 ppm, 2 ppm, 4 ppm and 8 ppm), was added with methanol and AlCl
3. Next, it was incubated and measured its absorbance at 510 nm of wavelength.Sample Testing
Some samples were weighed and dissolved in the methanol. Next,

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the absorbent was added to remo

ve the undesired compound. The absorbent contains the mixture of Al, Mg, SO4, and Si adding with sodium acetate as the buffer. Then, the samples were centrifuged for 10 minutes at 4500 rpm, added with methanol and AlCl3, incubated, and measured its absorbance at 510 nm of wavelength.Flavonoid
Analysis using LC-MS/MSThe fig fruit fermentation result was analyzed qualitatively using LC-MS/

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MS Thermo Scientific TSQ Quantum Access Max Type Tr

iple Quadropole, with hyper sile Gold 1.9 mm x 2.1 mm x 50 mm columns and mobile phase A containing 0.1%

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of formic acid in the water, phase B containing 0.1% of formic acid

in the methanol.Data Analysis

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The qualitative data were analyzed descriptively, whereas the quantitative data were analyzed usin

g the ANOVA. The differences between groups were tested using the Turkey HSD testing.RESULT
SFig Fruit Juice Fermentation
All lactic acid bacteria used as starts in this research can ferment the fig fruit juice, indicated by the fig fruit juice organoleptic and pH changes, as in the table
1.Flavonoid Identification
The flavonoid identification by the dye reaction shows that both in the fig fruit juice before and after the fermentation using some starter bacteria, the flavonoid compound can be identified
(Table

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2).Total Flavonoid Content
The identification result becomes the basis to conduct the Total Flavonoid Content

(TPC) determination of fig fruit juice and fermented fig fruit juice, as can be seen in figure 1.The TFC determination result shows that the flavonoid concentration of fig fruit juice experiences a decrease after the

fermentation process. Before the fermentation, the total flavonoid content of fig fruit juice is 82.59 ppm, whereas, after the fermentation, there is a decrease of 32 - 50%. The fermentation using Lactobacillus acidophillus results

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in the total flavonoid content of 40.66 ppm, using L. bulgaricus results in the total flavonoid content of 47.58 ppm, using L. casei results in the total flavonoid content of 55.68 ppm, and using L. plantarum results in the total flavonoid content of 45.20 ppm. The highest total flavonoid content afte

r the fermentation is resulted by the fermentation using Lactobacillus casei, but it does not significantly different with the other fermentation results using the other starter bacteria.LC-MS/MS Analysis
In this research, the qualitative testing using LC-MS/MS also was conducted to identify the flavonoid compounds on the fig fruit, as catechin, epicatechin, and rutin. The qualitative analysis

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result of LC-MS/MS can be seen in the figure 2.Based on the analysis result of LC-MS/MS,

the detected flavonoid, before and after the fermentation, is rutin with the absorbance area according to what is presented by the picture, whereas catechin and epicatechin cannot be detected. Based on the absorbance area obtained from the result analysis of LC-MS/MS, it was used to determine the relative percentage of rutin on the fig fruit juice and fermented fig fruit juice. The result can be seen in the table 3.Based on the relative percentage determination
(table 3), can be understood that after the fermentation, the relative percentage of rutin is higher than the percentage before the fermentation. It shows that qualitatively, the existence of rutin on the fermented fig fruit juice is higher than the existence of rutin before the fermentation. The increasing existence of rutin reaches 100 - 150%.DISCUSSION
The lactic acid bacteria activity causes the acid production in the fermentation result, which was indicated by the color change and pH decrease into more acid. It shows that the lactic acid bacteria used as the starters capable of using

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sugar in the fig fruit juice as a substrate for their growth. The use of sugar in the fig fruit

juice by the starter bacteria can cause the deconstruction of glycoside binding between the flavonoid and sugar releasing the flavonoid in the form of aglycone. The sugar group removal from the glycoside was conducted under the purpose for the bioavailability and/or plant functional flavonoid (Lee et al., 2015). The flavonoid existence can be identified by the dye reaction, as we seen in tabel 2. It shows qualitatively that the various lactic acid fermentation can maintain the flavonoid of fig fruit juice.The differences of flavonoid content in the fermentation can be affected by the strain used (Filannino et al., 2016). The decrease of total flavonoid content of fig fruit juice fermentation is different from the other studies mentioning that the fermentation can increase the flavonoid content, as on the fermentation of Chinese cabbage (Sun et al., 2009), black soybean (Juan & Chou, 2010), soybean seed (Singh et al., 2010), Avena sativa L. (Cai et al., 2011), cheonggukjang soybean (Cho et al., 2011), Graptopetalum paraguayense E. Walther (Wu et al., 2011), apple juice (Ankolekar et al., 2012), barley and wheat seeds (Hole et al., 2012), Houttuynia cordata (Kwon & Ha, 2012), okra (Abelmoschus esculentus) seed (Adetuyi & Ibrahim, 2014) and jaruk tigarun (Nazarni et

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al., 2016).The increase of flav

onoid content must be able to occur due to the microbial enzymes, as glucosidase, amylase, cellulose, tannase, esterase, invertase or lipase, produced during the fermentation that can hydrolyze the glucoside and break down the plant cell wall or starch. The enzymes play a role in the disintegration of cell wall matrix resulting in the flavonoid extraction (Hur et al., 2014). Another mechanism is during the fermentation, β-glucosidase from the microbes can also hydrolyze the phenolic and flavonoid. L. plantarum was reported to have a strong glucosidase activity. The active compounds, which experience increase, are predicted to be converted from the enzymatic cleavage on the appropriate glucosidase (Duenas et al., 2005)

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.The increase of flavonoid content of

fermented soybean using Bacillus pumilus HY1 is a result of esterase and tannase

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activity of lactic acid bacteria during the fermentat

ion (Cho et al., 2011). This phenomenon shows the possibility of metabolite conversion that can be stimulated through the fermentation. Based on this result, some flavonoids are expected can be degraded during the fermentation and/or resulted from the phenolic degradation (Rodriguez et al., 2009).Yet, there are
other studies mentioning that there is a decrease of flavonoid content after the fermentation, as on the tea

fermentation caused by the oxidation of flavonoid hydroxyl group (Winardi, 2010) and on the leaf extract of fermented Artocarpus communis (Ie & Uf,

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2016). The flavonoid content of the

sprout culture of Orthosiphon aristatus fermentation in vitro using L. plantarum also

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experienced a decrease after 24 hours of fermentation. The higher decrease occurred after

24 hours fermentation using L. acidophillus. Yet, the increase of flavonoid content occurred on the fermentation result using both of the bacteria with the longer fermentation time of 48 and 72 hours (Hunaefi et al., 2012).The decrease of flavonoid content can be caused by the temperature and pH. The increase of base temperature and pH
can affect the flavonoid degradation (Srivastava & Gupta, 2009). Besides, the processing can also decrease the flavonoid content depending on the method used (Kumar & Pandey, 2013).Based on the several factors causing

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the decrease of flavonoid content, it is expected that the decrease of flav

onoid of fig fruit juice fermentation was caused by the temperature. Before the fermentation, the fig fruit juice was pasteurized at 72'C, and in the fermentation, the temperature was 37'C. Besides the temperature, the other factor is the fermentation time. The fig fruit juice was fermented for 24 hours, whereas if it corresponds to the research of Hunaefi et al. (2012), the increase of flavonoid content occurred at the fermentation time of 48 and 72 hours. It is supported by the finding where it was reported that the flavonoid glycoside can be metabolized in the fermentation in vitro for 72 hours using the human fecal microflora (Justesen et al., 2000). Therefore, in order to understand the increasing possibility of flavonoid content of fig fruit juice, the further research on the variation of fermentation time until 72 hours is required.LC-MS/MS was conducted to identify the flavonoid compounds on the fig fruit, as catechin, epicatechin, and rutin. It is according to the research of Veberic et al. (2008) that the compounds have been identified on the dried fig fruit. Detected compound on the LC-MS/MS results is affected by the high and low of compound content on the fig fruit juice. The rutin can be detected presumably because it occurs in a higher content than the other compounds. It occurs in the highest concentration of other tested compounds (until 28.7

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mg/100 g), whereas the catechin occurs until 4.03 mg/100 g and t

he epicatechin occurs until 0.97 mg/100 g (Veberic et al., 2008).Several studies mentioned on the increase of rutin concentrations after the fermentation. It increased 1.9 times on the fermentation of Houttuynia cordata (Kwon and Ha, 2012). It also increased on the wheat fermentation with various of increase on each of dissolvent used, as ethanol (11.38%), acetone (9.17%) and water (128.35%) (Zhang et

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al., 2012).Based on the explanation above, it can b

e conclude that lactic acid fermentation using Lactobacillus acidophillus, L. bulgaricus, L. casei and L. plantarum

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affects the total flavonoid content of fig fruit juice. After the fermentation, there is a decrease in the total flavonoid content. The

decrease is predicted due to the temperature effect on the fermentation process and less fermentation time. The detected flavonoid, in both before and after the fermentation, through LC-MS/MS is rutin, with the relatively high percentage after the fermentation. It shows that the fermentation can increase the availability of rutin.

ACKNOWLEDGEMENT
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Service - the Directorate of General of Research and Development Reinforcement - the Ministry of Research, Technology and Higher Education of the Republic of Indonesia

that has funded this research through the Novice Lecturer Research Grant Program.Table 1. Organole
ptic and pH of Fig Fruit JuiceSample
Color
Aroma
Taste

pH
Non Fermented
Brown
Fig
Sweet
4.52
Fermented with Lactobacillus acidophillusLight brown
Fermentation
Sour
3.38
Fermented with Lactobacillus bulgaricusLight brown
Fermentation
Sour
3.37
Fermented with Lactobacillus caseiLight brown
Fermentation
Sour
3.37
Fermented with Lactobacillus plantarumLight brown
Fermentation
Sour
3.31
Table 2. Flavonoid Identification of Fig Fruit Juice
Sample
Flavonoid
Non Fermented
+
Fermented with Lactobacillus acidophillus+
Fermented with Lactobacillus bulgaricus+
Fermented with Lactobacillus casei+
Fermented with Lactobacillus plantarum+
Table 3. Relative Percentage of Rutin of Fig Fruit JuicesSample
Relative Percentage (%)
Non Fermented
100
Fermented with Lactobacillus acidophillus201.85
Fermented with Lactobacillus bulgaricus254.93
Fermented with Lactobacillus casei247.12
Fermented with Lactobacillus plantarum220.25
Figure 1. Total Flavonoid Content of Fig Fruit Juices. NF=Non Fermented; Fla= Fermented with Lactobacillus acidophillus; 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%.B
A
C
D
E
Figure 2. LC-MS/MS result of fig fruit juices
.A= Non Fermented, B= Fermented with Lactobacillus acidophillus, C= Fermented with Lactobacillus bulgaricus, D= Fermented with Lactobacillus casei, E= Fermented with Lactobacillus plantarum.17

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