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Original Article

The effect of lactic acid fermentation on fig (*Ficus carica*) fruit flavonoid

Ernanin Dyah Wijayanti and Nur Candra Eka Setiawan
Akademi Farmasi Putra Indonesia Malang

Abstract

Fig fruit contains a quite high flavonoid that supports the use of it for several disease 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 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.

Keywords: *Lactobacillus,* LC-MS/MS

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Introduction

Fig fruit is one of the fruits mentioned 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 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 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. 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, Ca^{2+}-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 β-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

* Corresponding Author:
Ernanin Dyah Wijayanti
Akademi Farmasi Putra Indonesia Malang Jl. Barito, No 5 Malang, 65123
Phone: 0341-491132 Fax: 0341-485411
e-mail: nanin.wijayanti@gmail.com

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

Materials

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 milk products.

Fig Fruit Juice Fermentation

Dried fig fruit was blended with water (1:5). The juice obtained was filtered and 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 Determination

Quercetin (Nacalai Tesque, Inc.) 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.

Some samples were weighed and dissolved in the methanol. Next, the absorbent was added to remove the undesired compound. The absorbent contains the mixture of Al, Mg, SO_4, and Si adding with sodium acetate as the buffer. Then, the samples were centrifuged for 10 minutes at 4500 rpm, added with methanol and $AlCl_3$, incubated, and measured its absorbance at 510 nm of wavelength.

Flavonoid Analysis using LC-MS/MS

The fig fruit fermentation result was analyzed qualitatively using LC-MS/MS [Thermo Scientific TSQ Quantum Access Max Type Triple Quadropole], with hyper sile Gold 1.9 mm x 2.1 mm x 50 mm columns and mobile phase A containing 0.1% of formic acid in the water, phase B containing 0.1% of formic acid in the methanol.

Data Analysis

The qualitative data were analyzed descriptively, whereas the quantitative data were analyzed using the ANOVA. The differences between groups were tested using the Turkey HSD testing.

Results

Fig 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 2).

Table 1. Organoleptic and pH of Fig Fruit Juice

Sample	Color	Aroma	Taste	pH
Non Fermented	Brown	Fig	Sweet	4.52
Fermented with *Lactobacillus acidophillus*	Light brown	Fermentation	Sour	3.38
Fermented with *Lactobacillus bulgaricus*	Light brown	Fermentation	Sour	3.37
Fermented with *Lactobacillus casei*	Light brown	Fermentation	Sour	3.37
Fermented with *Lactobacillus plantarum*	Light 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

Sample	Relative Percentage (%)
Non Fermented	100
Fermented with *Lactobacillus acidophillus*	201.85
Fermented with *Lactobacillus bulgaricus*	254.93
Fermented with *Lactobacillus casei*	247.12
Fermented with *Lactobacillus plantarum*	220.25

Total Flavonoid Content

The identification result becomes the basis to conduct the Total Flavonoid Content (TFC) 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 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 after 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.

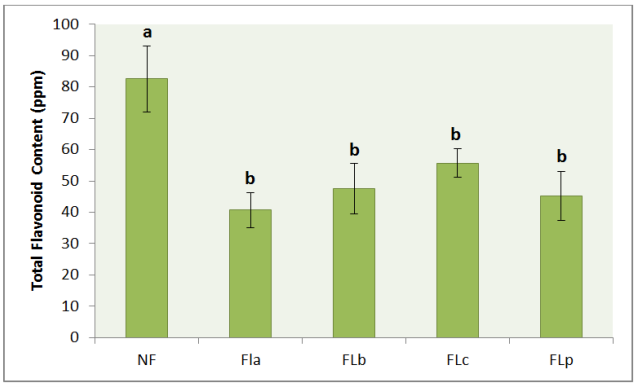
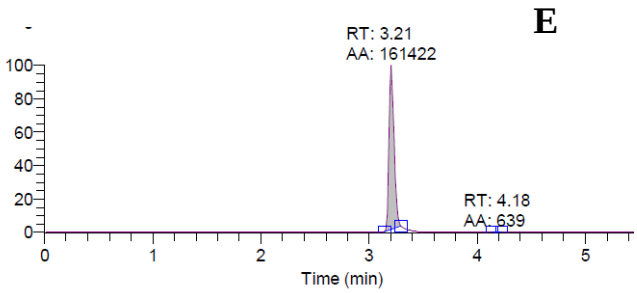


Figure 1. Total Flavonoid Content of Fig Fruit Juices. NF=Non Fermented; Fla= Fermented with *Lactobacillus acidophillus*; FLb= Fermented with *Lactobacillus bulgaricus*; FLc= Fermented with *Lactobacillus casei*; FLp= Fermented with *Lactobacillus plantarum*. The different alphabetical notations show the significant differences based on the Tukey HSD testing with the confidence interval of 95%.



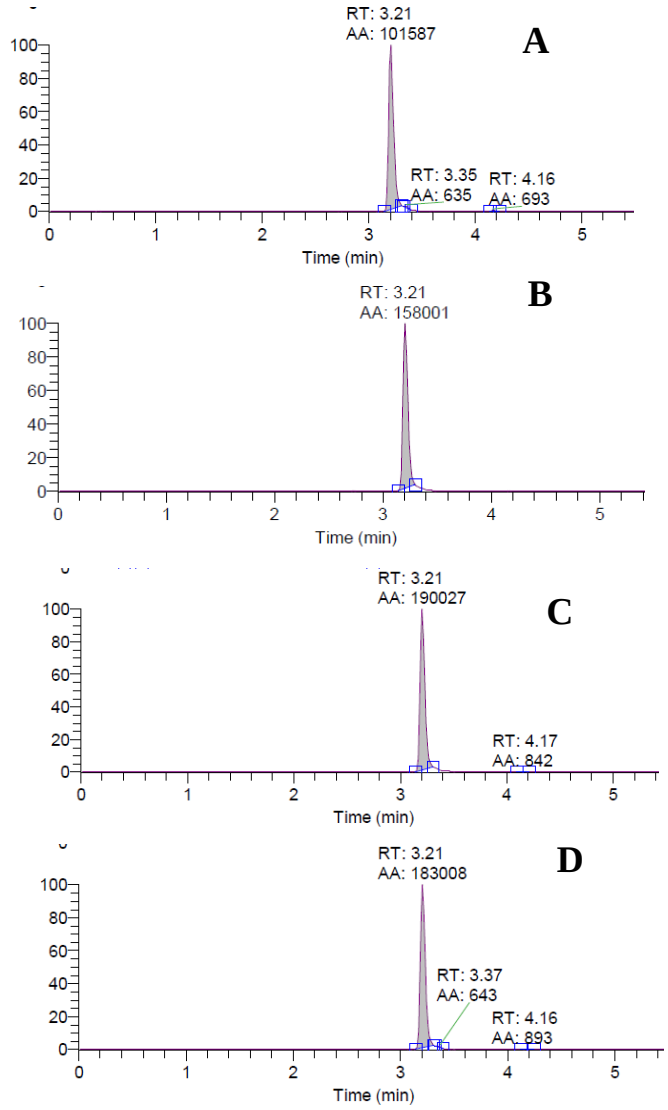


Figure 2. LC-MS/MS result of fig fruit juices.A= Non Fermented, B= Fermented with *Lactobacillus acidophillus*, C= Fermented with *Lactobacillus bulgaricus*, D= Fermented with *Lactobacillus casei*, E= Fermented with *Lactobacillus plantarum*.

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, whether as catechin, epicatechin, rutin or not. The qualitative analysis 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, was rutin with the absorbance area according to what was 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 showed that qualitatively, the existence of rutin on the fermented fig fruit juice was higher than the existence of rutin before the fermentation. The increasing existence of rutin reached 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 showed that the lactic acid bacteria used as the starters capable of using 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 table 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 was different from the other studies mentioning that the fermentation could 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* (Cai *et al*., 2011), cheonggukjang soybean (Cho *et al.*, 2011), *Graptopetalum paraguayense* (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 al.*, 2016).

The increase of flavonoid 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 played 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 also can hydrolyze the phenolic and flavonoid. *L. plantarum* was reported to have a strong glucosidase activity. The active compounds, which experience increase, was predicted to be converted from the enzymatic cleavage on the appropriate glucosidase (Duenas *et al.*, 2005).

The increase of flavonoid content of fermented soybean using *Bacillus pumilus* HY1 was a result of esterase and tannase activity of lactic acid bacteria during the fermentation (Cho *et al*., 2011). This phenomenon showed the possibility of metabolite conversion that can be stimulated through the fermentation. Based on this result, some flavonoids were expected can be degraded during the fermentation and/or resulted from the phenolic degradation (Rodriguez *et al*., 2009).

Yet, there were, 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, 2016). The flavonoid content of the sprout culture of *Orthosiphon aristatus* fermentation in vitro using *L. plantarum* also 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 could 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 could also decrease the flavonoid content depending on the method used (Kumar & Pandey, 2013).

Based on the several factors causing the decrease of flavonoid content, it was expected that the decrease of flavonoid 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 corresponded to the research of Hunaefi *et al*. (2012), the increase of flavonoid content occurred at the fermentation time of 48 and 72 hours. It was 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 was required.

LC-MS/MS was conducted to identify the flavonoid compounds on the fig fruit, as catechin, epicatechin, and rutin. According to Veberic *et al.* (2008), the compounds have been identified on the dried fig fruit. Detected compound on the LC-MS/MS results was affected by the high and low of compound content on the fig fruit juice. The rutin could be detected because it occured in a higher content than the other compounds. It occured in the highest concentration (until 28.7 mg/100 g), whereas the

catechin and the epicatechin were 4.03 mg/100 g and 0.97 mg/100 g, respectively (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 & 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 al*., 2012).

Based on the explanation above, it could be conclude that lactic acid fermentation using *Lactobacillus acidophillus, L. bulgaricus, L. casei* and *L. plantarum* affected the total flavonoid content of fig fruit juice. After the fermentation, there was a decrease in the total flavonoid content. The decrease was 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 was rutin, with the relatively high percentage after the fermentation. It showed that the fermentation colud increase the availability of rutin.

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