1st HSIC

Theme: "Evidence-based Practice in Health Science"

Malang, East Java - Indonesia
4th -5th October 2017

1st HEALTH SCIENCE INTERNATIONAL CONFERENCE

UMM
UNIVERSITAS MUHAMMADIYAH MALANG

SCHEDULE – Day 1

DATE: OCTOBER 4, 2017

VENUE: CONFERENCE HALL, SWISS-BELINN HOTEL, MALANG

TIME	AGENDA		
08.50 - 09.15	Opening Remark		
	1. Welcome dance		
	2. Opening		
	3. Indonesia Raya National Anthem		
	4. Sang Surya		
	5. Qur'an Recitation		
09.15 - 09.30	Welcome Speech by Rector of UMM		
09.30 - 09.45	Coffee break		
09.45 – 10.15	Keynote Speaker 1: Associate Professor Su-Ying Fang, PhD (The Application of Evidence Based Practice on Cancer Patient)		
10.15 – 10.55	Keynote Speaker 2: P. Yoyok Bekti P., M. Kep. Sp. Kom (Role of Community Nurses in Improving Carer's Ability In Caring Children With ARFID (Avoidant Restrictive Food Intake Disorder))		
10.55 – 11.30	Discussion		
11.30 – 12.30	Lunch Break		
12.30 – 13.10	Keynote Speaker 3: Prof. Dr. hab. N.med. Kinga Barowicz-Reutt (Neurosteroids and their neuroprotective action)		
13.10 – 13.50	Keynote Speaker 4: Professor Roland J. Pieters (Carbohydrate Based Drugs)		
13.50 – 14.15	Discussion		
14.15 – 15.00	Coffee break		
15.00 – 16.00	Oral presentation Session 1		
	R. Arjuna 1	R. Arjuna 2	R. Arjuna 3
	1. Engrid Juni A.	1. M. Rosyidul Ibad	1. Fuad Husain Akbar
	2. Sovia Aprina B.	2. Ani Sutriningsih	2. Fithria Dyah A. S.
	3. Ika Ratna	3. Fajar Rinawati	3. Supriyatiningsih
	4. Rasmidar Samad	4. Tutu April A.	4. Gisely Vionalita
16.00-17.00	Oral presentation Session 2		
	R. Arjuna 1	R. Arjuna 2	R. Arjuna 3
	1. Uswatun Chasanah	1. Kusuma Andriana	1. Fathiyah S.
	2. Dian Ermawati	2. Dwi Rahayu	2. Salim Haris
	3. Ernanin Dyah W.	3. Ririn Harini	3. Melia A.
	4. Oki Nugraha P.	4. Gita Sekar P.	4. Olena Pilipovich
17.00	Closing of Day 1		

SCHEDULE – Day 2

DATE: OCTOBER 5, 2017

VENUE: CONFERENCE HALL, SWISS-BELINN HOTEL, MALANG

TIME	AGENDA		
07.30 - 08.00	Registration		
08.00 – 09.15	Oral presentation Session 1		
	R. Arjuna 1	R. Arjuna 2	R. Arjuna 3
	1. Lilik Wijayanti	1 Zahid Fikri	1. Sih Ageng L.
	2. Yuliana Heri S.	2. Idola Perdana	2. Kolifah
	3. Siti Rofida	3. Faqih Ruhyanudin	3. Nungki M. Y
	4. Dian Yuliartha	4. Indah D. P	4. Nailis Syifa
	5. Ali Multazam		5. Henny Dwi S.
09.15 – 09.30	Coffee break		
09.30 - 10.45	Oral presentation Session 2		
	R. Arjuna 1	R. Arjuna 2	R. Arjuna 3
	1. Edi P.	1. Nur Lailatul M.	1. Reni Ilmiasih
	2. Ariani	2. Endang Wahyu	2. Sondang Sianturi
	3. Chrisnawati	3. Feriana Ira H.	3. Sunardi
	4. Nur Aini	4. Muthmainah	4. Abdul Muhith
		5. Tasnim	
10.45 – 11.45	Oral presentation Session 3		
	R. Arjuna 1	R. Arjuna 2	R. Arjuna 3
	1. Anggraini D.K	1. Henik Tri Rahayu	1. Sri Sunaring Ika
	2. Zaqqi U.	2. Aini Alifatin	2. Atika Yulianti
	3. Titin Wahyuni	3. Nuh Huda	3. Dimas Sondang
	4. Sherly Frida	4. Atyanti Isworo	4. Safun Rahmanto
11.45 - 12.00	Break		
12.00	Closing		
	1. Award of Best Oral Presenter		
	2. Closing by Vice Dean of Faculty of Health Science, University of Muhammadiyah Malang		

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Advances in Health Sciences Research (AHSR), volume 2
Health Science International Conference (HSIC 2017)

Effect of Lactic Acid Fermentation on Total Phenolic Content and Antioxidant Activity of Fig Fruit Juice (*Ficus carica*)

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ABSTRACT

Background: *Fig (Ficus carica) is used for a disease therapy due to its relatively complete nutrition and active compounds as sugar, mineral, vitamin, organic acid and phenolic compounds. A high phenolic compound in fig is related closely to its fruit activity as an antioxidant. Yet, its difficult for phenolic compound to be absorbed by the human body, so a fermentation is required.* ***Objectives:*** *This research aims to determine the effect of lactic acid fermentation on total phenolic content and antioxidant activity of fig fruit juice.* ***Method:*** *Fig fruit juice was fermented at 37°C for 24 hours using 4 types of starter bacteria as Lactobacillus acidophilus, L. bulgaricus, L. casei and L. plantarum. The total phenolic content testing was conducted using the spectrophotometry method using sulfanilic acid reagent. The antioxidant activity was tested using the spectrophotometry method using DPPH reagent.* ***Results:*** *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.* ***Conclusion:*** *Based on the results, 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 which has many benefits for the health. It is mostly used as an anti-cancer, in degenerative disease therapy, digestion problem, osteoporosis and infectious disease prevention due to its quite complete nutrition contents. That is why, this fruit is known as a "paradise fruit".

Some researchers have demonstrated various fig fruit contents to support its use for the society. Different parts of the 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 carbohydrates, sugar, minerals, vitamins, organic acids, and phenolic compounds (Soni et al. 2014).

Fig fruit contains high level of phenolic compounds to support its use as an antioxidant. Yet, mostly he phenolic compounds are difficult to be absorbed by the human body. The highest level of phenolic compounds is found in the vegetables, except flavonol which is found in the glycosylated form and glycosylation is affecting the absorption. To make the absorption easear, the hydrolysis by enzymes or microbes in the digestive tract is

required (Filannino et al. 2016). In line with the previous statement, the fig fruit contents require the hydrolysis processes, one of them is fermentation process.

Fermentation can increase the nutrition value in the foodstuffs. It can also increase the antioxidant activity in the fermented materials. Fermentation can increase the antioxidative activity by increasing the flavonoid release of plant-based food. Various biochemical changes occurred during the fermentation causing the nutrition component and anti-nutrition ratio changes influencing the product characteristics, as a bioactivity and digestibility (Zhang, Soccol & Pandey 2012).mThe glycoside conversion into the aglycone by the fermentation is the main principle of the antioxidative activity increase in the plant-based food (Hur et al. 2012).

The most applied fermentation in the foodstuffs is a lactic acid fermentation. This fermentation type uses lactic acid bacteria which safety and benefits are proven. *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 effect of lactic acid fermentation on total phenolic content and antioxidant activity of fig fruit juice.

METHOD

Materials

Dried fig fruit was purchased from the tin leaf tea producer (Kunta tea) in Gresik, East Java. *Lactobacillus acidophillus* and *Lactobacillus plantarum* bacterial cultures were purchased from the Microbiology Laboratory, Agricultural Product Technology Department, Agricultural Technology Faculty, Brawijaya University, Malang, East Java, while *Lactobacillus bulgaricus* and *Lactobacillus casei* cultures were isolated from the commercial fermented products.

Fig Fruit Juice Fermentation

Dried fig fruit was blended and diluted 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 to around 40°C. After that, the juice was added to 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%, H_2SO_4, 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 wavelength. The standard curve of phenol with the same procedure was made .

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, SO_4, 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 wavelength.

RESULT 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 was 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).

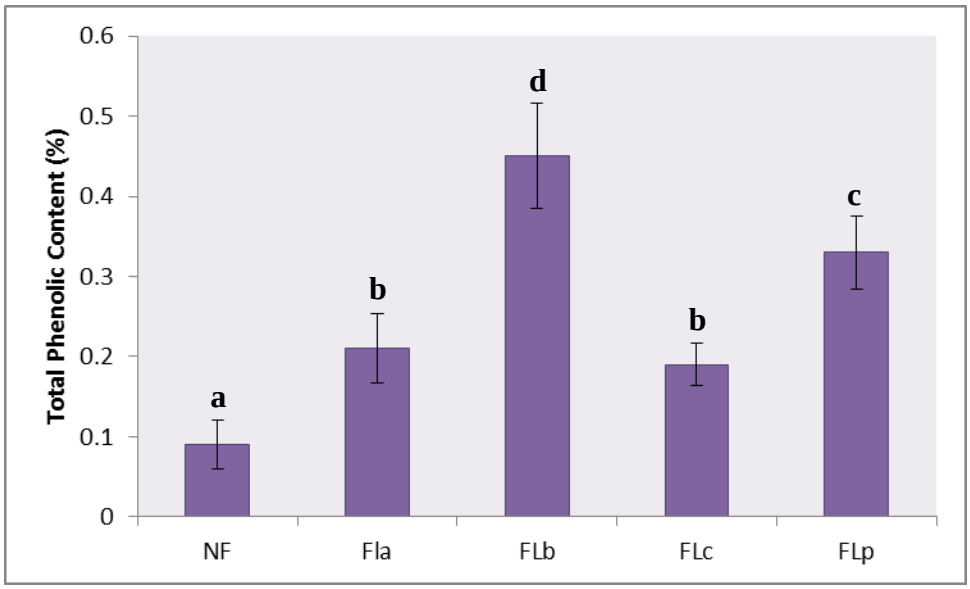

Figure 1 Total Phenolic Content of Fig Fruit Juices. NF=Non Fermented; Fla= Fermented with *Lactobacillus acidophillus*; FLb= Fermented with *Lactobacillus bulgaricus*; FLc= Fermented with *Lactobacillus casei*; FLp= Fermented with *Lactobacillus plantarum*. The different alphabetical notations show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

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. 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 the quality and nutritional value of food (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. It was stated that the fermentation process causes the microbial enzyme release producing higher chemical compounds from the plant as flavonoids, tannin, alkaloids, and phenylpropanoid (Nazarni

et al. 2016). The lactic acid bacteria existence in the fermentation contributes to the simple phenolic conversion and phenolic compounds depolymerization with the high molecular weight (Othman et al. 2009).

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 (Dueñas et al, 2005). It was also stated that the fermentation can induce the cell wall structural breakdown causing the bioactive compounds release and/or synthesis. During the fermentation, the bound phenol can be released enzymatically (Zhang, Soccol & Pandey 2012).

The increase 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 jaruk tigarun flowers (Nazarni et al. 2016), *Graptopetalum paraguayense* E. Walther (Wu, Su & Cheng 2011), *Echinacea spp.* (Rizzello & Coda 2013), and Malaysian herbal teas (Ibrahim, Mustafa & Ismail 2014). It was also reported that parts of plants experiencing the increase of total phenolic content after the fermentation (Ng et al. 2014).

Effect of Lactic Acid Fermentation on Antioxidant Activity

The lactic acid fermentation produces a various antioxidant activity of the fig fruit juice. 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. IC50 is defined as the antioxidant amount required for reducing 50% of DPPH absorbance from the early absorbance (Mishra, Ojha & Chaudhury 2012).

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. acidophillus* 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.

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. acidophillus* produces the average antioxidant activity. It refers to antioxidant activity power which 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 (Salusu et al. 2017).

The antioxidant activity change after the fermentation process can be affected by the starter bacteria used. It was 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. The fermentation process is supposed to increase the antioxidant activity (Zhang, Soccol & Pandey 2012). 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 (Marazza et al. 2009). 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. acidophillus* 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).

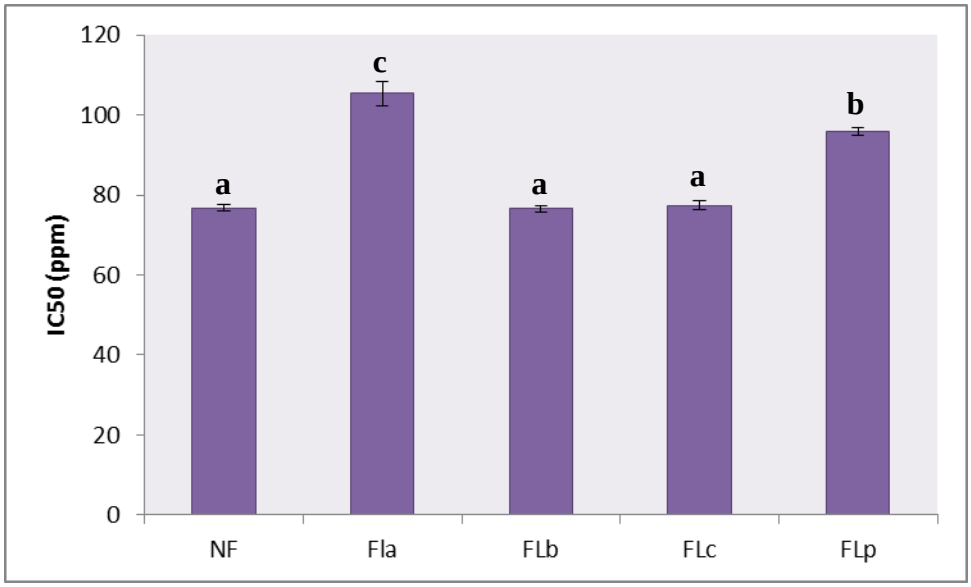

Figure 2 IC50 of Fig Fruit Juice. NF=Non Fermented; Fla= Fermented with *Lactobacillus acidophillus*; FLb= Fermented with *Lactobacillus bulgaricus*; FLc= Fermented with *Lactobacillus casei*; FLp= Fermented with *Lactobacillus plantarum*. The different alphabetical notations show the significant differences based on the Tukey HSD testing with the confidence interval of 95%.

The antioxidant activity of fig fruits juice can be produced by the secondary metabolite compounds in fig fruit. There was a research stated that in the dried fig fruit there are phenolic (10.90 µg GAE/mg sample), flavonoid (2.75 µg CE/ mg sample), the alkaloid (9.6%) and saponin (0.59%) (Aksoy et al. 2013). Generally, 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 plays 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 (Aksoy et al. 2013).

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, 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 groups on the whole molecule as double bond and its conjugation towards the

hydroxyl and ketone groups (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 *Thermopsis turcica* (Aksoy et al. 2013), buckwheat (*Fagopyrum esculentum* Moench) (Sun & 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 Sinensisis (Li et al. 2009), and *Solanum tuberosum* (Hesam et al. 2012). 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). Another study also 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 was demonstrated by the extract of *Centella asiatica*, yet it produces the second highest antioxidant activity, both in the DPPH and SOD methods (Rafat, Philip & Muniandy 2010).

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 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

The lactic acid fermentation is able to increase the total phenolic content significantly and preserve the antioxidant activity, which is classified as a active category. The increase of the 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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Faculty of Health Science - University of Muhammadiyah Malang
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