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Author Name Islamudin Ahmad, Desta Andriyani, Christoper Gunawan, Nisrina Dhiah Fauziah, Sutriyo, Abdul Mun'im		Title of the paper Effects of Food Processing on Resveratrol and Total Phenolic Content in Melinjo (<i>Gnetum gnemon</i> L.) Seeds	Journal Name Pharmacognosy Journal	
Author email			PDF file name PJ-61-18-QC-RD	MS Number pages
Comment Number (As marked in Galley Proof)	Page Number, Line Number	As in Galley Proof	Modification to be done	Remarks
1.	Page 74, line number 32 (right column)	".....peanut. ²⁶ including grapes,....."	".....peanut, ²⁶ including grapes,....."	revision
2.	Page 74, line number 32 (right column)	"...Zupancic <i>et al.</i> ²⁷ this paper....."	"...Zupancic <i>et al.</i> , ²⁷ this paper....."	revision
3.	76, (References, point 23)	23. Resurreccion, AVA, Rudolf JL, Phillips RD, Chinnan M, Method form enhancing resveratrol content of peanut compositions. Journal name missing??? 2010; 2(12):12-15. Doi:10.1016/j.(73)	23. Resurreccion, AVA, Rudolf JL, Phillips RD, Chinnan M, Method form enhancing resveratrol content of peanut compositions. <i>United State Patent</i> . 2010; Patent No.: US 7,666,455 B2	replace
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Original Article

<RH> Ahmad, *et al.*: Effects of food processing on total phenolic and resveratrol content in Melinjo seeds

Effects of Food Processing on Total Phenolic and Resveratrol Content in Melinjo (~~*Gnetum gnemon*~~*Gnetum gnemon* L.) Seeds

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ABSTRACT

Objective: *Trans*-resveratrol is a phenolic compound in the stilbene group present in Melinjo (~~*Gnetum gnemon*~~*Gnetum gnemon* L.) seeds. In Indonesia, these seeds are consumed by roasting, frying (i.e., emping), and boiling. This study aimed to evaluate the effects of food processing on resveratrol and total phenolic compound levels in melinjo seeds. **Methods:** Melinjo seed was roasted at 145°C for 10 and 20 min, boiled at 100°C for 5, 15, and 30 min, and fried at 160-170°C for 2 and 4 min. Samples were refluxed with 96% ethanol. The determination of resveratrol levels was performed using high-performance liquid chromatography, and total phenolic content was determined using Folin-Ciocalteu method. **Results:** Based on the results, it was found that resveratrol and total phenolic content levels in Melinjo powder decreased from 36% to 10% (resveratrol) and 15% to 4% (total phenolic) after 20 min of roasting, and in fried emping, it was 60%, 68%, and 92% (resveratrol) and 41%, 45%, 97% (total phenolic) after 5, 15, and 30 min of boiling, respectively. **Conclusion:** There were significant changes in resveratrol levels and total phenolic content in Melinjo seeds before and after various cooking processes (i.e., roasting, boiling, and frying).

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Key Words: Food processing, ~~*Gnetum gnemon*~~*Gnetum gnemon* L., Resveratrol level, ~~T~~total phenolic content.

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INTRODUCTION

Melinjo (~~*Gnetum gnemon*~~*Gnetum gnemon* L.) or in Sundanese is known as “Tangkil,” is a species of *Gnetum* (Gymnospermae) native to tropic Asia, Melanesia, and West Pacific. Melinjo seeds have been consumed by the community as vegetables and as a snack in the form of Melinjo chips. The seed was reported to demonstrate some pharmacological activity, such as antioxidants,¹ anti-cancer,² anti-coagulant,³ hyperuricemia,⁴ HMG-CoA reductase,⁵ anti-obesity,⁶ and angiotensin-converting enzyme inhibitory.⁷

The seeds are rich-in stilbenes, such as resveratrol, which occurs as two isomers: *cis*-resveratrol and *trans*-resveratrol. Both isomers have different biological activity (such as antioxidant, HMG-CoA reductase, antihypertension, and so on). However, effects produced by *trans*-resveratrol are stronger than the results produced by *cis*-resveratrol.⁸ It has also been found that resveratrol stability is affected by light and high temperatures.⁹ In Indonesia culinary practices, Melinjo seeds are often as snack food ingredient and are processed into sundried, flat cakes called emping, which are fried in cooking oil before consumption.¹⁰ The seeds are also consumed as an additional ingredient in Indonesian soup (i.e., *Sayur Asem*) or boiled with vegetables and spices. As a foodstuff, Melinjo seeds have also been reported to have no toxic effects when consumed over long periods of time.¹¹

Food processing that involves heat, including roasting, baking, boiling, and frying, affect chemical compounds in some plants and foodstuffs. Previous studies have shown that the roasting process alters structural and chemical compounds by decreasing liquid content, modifying lipids, and causing discoloration and flavor changes.¹² Additionally, one study reported that the resveratrol level in roasted peanuts was less than in raw peanuts,¹³ while another study reported that *trans*-resveratrol levels in raw blueberries were higher than in roasted blueberries.¹⁴ However, research of food processing on the concentration of resveratrol and total phenolic content in Melinjo seeds has not been reported. The current study was conducted to determine the food processing effect by roasting, boiling, and frying and its duration on Melinjo seeds on *trans*-resveratrol and phenolic content.

MATERIALS AND METHODS

Plant Material

Melinjo (~~*Gnetum gnemon*~~*Gnetum gnemon* L.) seeds were achieved from Pandeglang traditional market, Banten Province, Indonesia and authenticated at the Herbarium Bogoriense, Bogor, and West Java, Indonesia. The voucher specimen was deposited at Herbarium of Pharmacognosy–Phytochemistry, Faculty of Pharmacy, Universitas Indonesia, Depok, and West Java, Indonesia.

Chemical Materials and General Equipment

The chemicals were used in this study, such as ethanol, aqua DM, ethyl acetate, n-hexane were purchased from PT. SmartLab Indonesia, Indonesia. Acetonitrile, acetic acid, Folin-Ciocalteu reagent, Sodium carbonate, methanol pro analysis were obtained from PT. Merck, Germany via PT Elo Karsa Utama, Indonesia. The equipment were used including oven vacuum (Jisico, Korea), rotary vacuum evaporator (Buchi, Switzerland), high-performance liquid chromatography (HPLC) (Shimadzu SPD-20A, Japan), and microplate reader (VersaMax, USA).

Food Processing

Fried Emping

Raw emping was fried in cooking oil for 2 and 4 min at 160–170°C and the oil removed by refluxing with n-hexane. Both raw and the fried emping were ground into powder.

Roasted Melinjo seed powder

Melinjo seeds were peeled to remove the outer shell and to expose the yellowish white inner seed before being ground into powder with a blender. This powder was inserted into a drying cabinet equipped with lights for seven days; after which time, the powder was removed for use in the experiment. Melinjo seed powder with and without eggs was roasted in an oven at 145°C for 10 and 20 min and cooled to room temperature.

Boiled Melinjo Seeds

Melinjo seeds with and without skin were boiled in water at 100°C at a ratio of 1:5 (seeds: water) for 5, 15, and 30 min before removal and milling to powder.

Extract Preparation

All samples were refluxed using 96% ethanol (Merck, Germany) for 1 h. The same procedure was repeated three times. The solvent was removed using a rotary vacuum evaporator at 50°C, and the samples were dried using an oven vacuum at 50°C.

Determination of Resveratrol Content Using High-Performance Liquid Chromatography (HPLC)

Resveratrol levels were determined using HPLC according to the Suoto method.¹⁵ with modification. Reverse phase HPLC was performed using a C18 column (5 µm particle size, 4.6

x 150 mm, Zorbax). The mobile phase was conducted in acetonitrile: water (25:75) solution (Merck, Germany), with the pH adjusted to 3 using acetic acid. Ultraviolet detection was performed at 306 nm, the flow rate was determined to be 1 ml/min, and the injection volume was determined to be 20 µl.

Determination of Total Phenolic Content (TPC)

TPC was determined using the Folin–Ciocalteu (F-C) method. Each sample weighed 20 grams and was dissolved in a methanol aqua demineralization mixture diluted to 400 ppm; 20 µL of the solution was piped and inserted into a 96-well microplate with each sample done in triplicate. Then, 100 µL of an F-C reagent was added, and the mixture was shaken for 1 min and incubated for 4 min. Then, 75 µL of Na₂CO₃ was shaken for 1 min and incubated for 120 min. After the incubation process, the absorbance of the solution was measured using a microplate reader.^{16,17}

RESULTS AND DISCUSSION

In present study, the influence of food processes (including roasting, boiling, and frying) on the difference of resveratrol and polyphenolic content of Melinjo seeds has been done. After the food processes, then each sample was macerated using 96% ethanol. The obtained extract was analyzed resveratrol levels and total polyphenolics content.

Determination of Resveratrol Content

As can be seen in **Figure 1**, resveratrol level was calculated using a calibration curve and the regression equation $y = 167959x + 2341.5$ with an r value = 0.9991. Retention time was used to identify resveratrol in a sample. The peak of the *trans*-resveratrol standard is shown at an 8-min retention time.

Table 1 shows an increasing level of resveratrol in Melinjo seed powder without egg (36%) compared to powder with egg (12%) after roasting for 10 min. This result was similar to previous research conducted by Rudolf (2003),¹⁸ which showed that resveratrol levels in nuts increased after the roasting and has been patented since 2010.¹⁹ Resveratrol content decreased by 10% in the sample without eggs and by 24% in the sample with eggs after baking for 20 min, possibly due to stilbene compounds, particularly resveratrol, being damaged.¹³ Protein in eggs is known to easily

denature at high temperatures, with the denaturation product forming aggregates, including ovalbumin.²⁰ These protein aggregates are water insoluble; thus, high temperatures decrease protein solubility.²¹

The resveratrol level of fried emping is shown in **Table 2** and was found to decrease after the frying process, which is similar to previous results for *Vaccinium myrtillus* L. and *Vaccinium corymbosum* L. after baking.¹⁴ Another study reported that the resveratrol level in raw peanuts was higher than in roasted peanut.²² The resveratrol content in boiled melinjo (**Table 1**) decreased over time because of the instability of resveratrol. This instability was investigated by Zupančič *et al.* (2015),²³ who found that resveratrol stability was dependent on temperature; when resveratrol was heated to over 30°C, its isomerization form changed from *trans*-resveratrol to *cis*-resveratrol. Additionally, as the temperature increased resveratrol degraded into a degradation product. To test the results, the data were analyzed using statistical package for the social sciences (SPSS) using a one-way analysis of variance (ANOVA) method, which indicated that changes in resveratrol level were significant ($p < 0.05$).

Determination of Total Phenolic Content (TPC)

As can be seen in Table 2, demonstrated TPC in roasted Melinjo seed powder, including changes when eggs were included ($p < 0.05$). These results supported the results obtained using HPLC. Xu and Chang (2008) found that temperature treatments, such as heating or roasting, of plants likely evaporated water at an intracellular level, triggering chemical reactions resulting in cell structure changes and increasing the availability of phenolic compounds in plant matrices.²⁴ Similarly, Yang *et al.* (2011) reported that the TPC of beans roasted for 5 min increased compared to raw beans.²⁵ and Hečimović *et al.* (2011) also reported the comparative study of polyphenols and caffeine in different coffee varieties affected by the degree of roasting.²⁶ The change in TPC in fried emping was also significant ($p < 0.05$), based on a one-way ANOVA and Tukey's post hoc analysis. A decrease was expected because the polyphenol group is heat sensitive, which includes resveratrol. Previous studies reported that the TPC in melinjo seeds decreased during the boiling process,¹ which was supported in the presents study; the TPC in boiled melinjo seeds decreased with boiling time (**Table 2**; $p < 0.05$). This decrease occurred because the heating process in boiling made polyphenolic molecules unstable and ruptured molecule bonds.

CONCLUSIONS

Based on the results above, Effects of food processing on concentration of resveratrol and total phenolic content in Melinjo (~~*Gnetum gnemon*~~*Gnetum gnemon* L.) seeds has been performed. There were significant changes in resveratrol levels and total phenolic content in Melinjo seeds before and after various cooking processes (i.e., roasting, boiling, and frying). The difference of resveratrol and polyphenolic content in each food processing of Melinjo seeds is preliminary data for further study and its effect on pharmacological effects.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

All author declared that have no conflict of interest.

ABBREVIATIONS USED

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TABLES AND FIGURES

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Figure 1: (A) Chromatogram of standard trans-resveratrol; (B) Chromatogram of sample.

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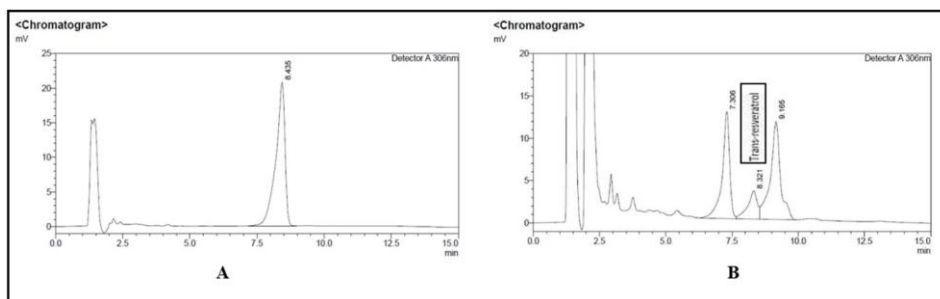


Table 1: Resveratrol content in melinjo powder, emping, and melinjo seeds.

Sample	Minutes	Resveratrol Content (mg/g)	Changes (%)	
Melinjo powder without egg	0	0.1025 ±		
	10	0.0075	36,00	↑
	20	0.1397 ±	10,00	↓
		0.0076 *		
		0.1262 ±		
		0.0090 *		
Melinjo powder with egg	0	0.0870 ±		
	10	0.0104	12,00	↑
	20	0.0973 ±	24,00	↓
		0.0147		
		0.0738 ±		
		0.0076 *		
Raw emping	0	0.123 ±		
		0.002		
Fried emping	2	0.095 ±	22.76	↓
		0.002*		
Fried emping	4	0.085 ±	30.89	↓
		0.002*		
Melinjo seeds without		1.028		
		0.375	63.81	↓
		0.113	68.42	↓
		0.008	91.67	↓

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skin, boiled			
Melinjo seeds with skin, boiled		1.259 0.412 0.140 0.008	66.4 64.29 93.33



* Significant change.

Table 2: Total Polyphenolics Content in melinjo powder, emping, and melinjo seeds.

Sample	Minutes	Total Phenolic Content (mg GAE/g)	Changes (%)
Melinjo powder	0	10.6436 ± 0.2444	
without egg	10	12.2379 ± 0.3543*	15.00
	20	11.6923 ± 0.6070*	4.00
Melinjo powder	0	7.2126 ± 0.4675	



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with egg	10	7.2482 ±		↑
	20	6.2451 ± 0.2226*	14.00	↓
Raw emping	0	99.621 ± 0.63		
Fried emping	2	84.829 ± 1.013*	14.85	↓
Fried emping	4	56.794 ± 1.14*	42.99	↓
Melinjo seeds without skin, boiled	0	45.312		
	5	26.629	40.79	↓
	15	14.799	44.76	↓
Melinjo seeds with skin, boiled	0	48.456		
	5	29.425	36.43	↓
	15	18.758	36.09	↓
	30	0.491	97.34	↓

* Significant change