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

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Submission date: 02-Sep-2019 08:09AM (UTC-0700)
Submission ID: 1166261624
File name: Phcogj_Vol_10_6.pdf (442.71K)
Word count: 4044
Character count: 22098

Pharmacogn J. 2018; 10(6):1096-1100
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
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Original Article

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

Islamudin Ahmad[1], Desta Andriyani[2], Christoper Gunawan[2], Nisrina Dhiah Fauziah[2], Sutriyo Sutriyo[3], Abdul Mun'im[2,*]

ABSTRACT

Objective: *Trans*-resveratrol is a phenolic compound in the stilbene group present in Melinjo (*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 *trans*-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 *trans*-resveratrol levels was performed using high-performance liquid chromatography (HPLC), and total phenolic content was determined using Folin-Ciocalteu method. **Results:** Based on the results, it was found that *trans*-resveratrol and total phenolic content levels in Melinjo powder decreased from 36% to 10% (*trans*-resveratrol) and 15% to 4% (total phenolic) after 20 min of roasting, and in fried empings, it decreased 60%, 68%, and 92% (*trans*-resveratrol) and 41%, 45%, 97% (total phenolic) after 5, 15, and 30 min of boiling, respectively. **Conclusion:** There were significant changes in *trans*-resveratrol levels and total phenolic content in Melinjo seeds before and after various cooking processes (i.e., roasting, boiling, and frying).

Key words: Food processing, Melinjo seeds, *Gnetum gnemon* L., Resveratrol levels, Total phenolic content.

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History

- Submission Date: 14-03-2018;
- Review completed: 28-06-2018;
- Accepted Date: 18-07-2018

DOI : 10.5530/pj.2018.6.186

Article Available online
http://www.phcogj.com/v10/i6

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INTRODUCTION

Melinjo (*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,[5-6] anti-obesity,[7] an d angiotensin-converting enzyme inhibitory.[8]

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.[9-10] It has also been found that resveratrol stability is affected by light and high temperatures.[11] 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.[12] 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.[13]

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.[14] Additionally, one study reported that *trans*-resveratrol level in roasted peanuts was less than in raw peanuts,[15] while another study reported that *trans*-resveratrol levels in raw blueberries were higher than in roasted blueberries.[16] However, research of food processing on the concentration of *trans*-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.

Cite this article: Ahmad I, Andriyani D, Gunawan C, Fauziah ND, Sutriyo S, Mun'im A. Effects of Food Processing on Resveratrol and Total Phenolic Content in Melinjo (*Gnetum gnemon* L.) Seeds. Pharmacog J. 2018;10(6):1096-100.

MATERIALS AND METHODS

Plant Material

Melinjo (*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. Smart Lab Indonesia, Indonesia. Acetonitrile, acetic acid, Folin-Ciocalteau 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 (Versa Max, 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[17] 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)

Trans-resveratrol levels were determined using HPLC according to the Suoto method.[18] 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–Ciocalteau (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 pipetted 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 Na_2CO_3 solution 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.[19-21]

RESULTS AND DISCUSSION

In present study, the influence of food processes (including roasting, boiling, and frying) on the difference of *trans*-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 *trans*-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 R value = 0.9991. Retention time was used to identify *trans*-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 *trans*-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),[22] which showed that *trans*-resveratrol levels in nuts increased after the roasting and has been patented since 2010.[23] *Trans*-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.[15] Protein in eggs is known to easily denature at high temperatures, with the denaturation product forming aggregates, including ovalbumin.[24] These protein aggregates are water insoluble; thus, high temperatures decrease protein solubility.[25]

The *trans*-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.[16] Another study reported that *trans*-resveratrol level in raw peanuts was higher than in roasted peanut,[26] including grapes, and may be one of the compounds responsible for the health benefits of red wine. Analytical methods for measuring resveratrol in wine and peanuts were adapted to isolate, identify, and quantify resveratrol in several cultivars of peanuts. Aqueous ethanol (80% v/v *Trans*-resveratrol content in boiled melinjo (Table 1) decreased over time because of the instability of resveratrol. This instability was investigated by Zupancic *et al.*,[27] this paper presents newly determined trans-RSV solubility and stability at various pH and temperatures, and the importance of such data for the studies of novel trans-RSV-loaded nanofibers. In acidic pH trans-RSV was stable, whereas its degradation started to increase exponentially above pH 6.8. Consequently, it is worthwhile to note that special consideration has to be dedicated to long dissolution testing or biological assays on cell lines in order to obtain relevant data. Measurements were done by validated

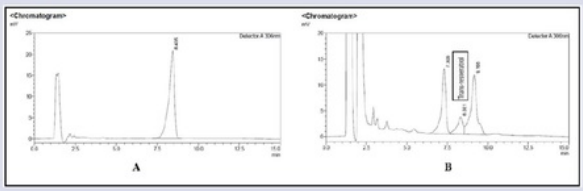

Figure 1: (A) Chromatogram of standard trans-resveratorl; (B) Chromatogram of sample.

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

Sample	Min	Resveratrol Content (mg/g)	Changes (%)
Melinjo powder without egg	0	0.1025 ± 0.0075	
	10	0.1397 ± 0.0076 *	36,00
	20	0.1262 ± 0.0090 *	10,00
Melinjo powder with egg	0	0.0870 ± 0.0104	
	10	0.0973 ± 0.0147	12,00
	20	0.0738 ± 0.0076 *	24,00
Raw emping	0	0.123 ± 0.002	
Fried emping	2	0.095 ± 0.002*	22.76
Fried emping	4	0.085 ± 0.002*	30.89
Melinjo seeds without skin, boiled	0	1.028	
	5	0.375	63.81
	15	0.113	68.42
	30	0.008	91.67
Melinjo seeds with skin, boiled	0	1.259	
	5	0.412	66.4
	15	0.140	64.29
	30	0.008	93.33

* Significant change.

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

Sample	Min	Total Phenolic Content (mg GAE/g)	Changes (%)
Melinjo powder without egg	0	10.6436 ± 0.2444	
	10	12.2379 ± 0.3543*	15.00
	20	11.6923 ± 0.6070*	4.00
Melinjo powder with egg	0	7.2126 ± 0.4675	
	10	7.2482 ± 0.3052*	0.50
	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
	30	0.421	97.17
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

UV/VIS spectroscopy, HPLC, and newly developed UPLC methods. Specificity was confirmed for HPLC and UPLC method, whereas UV/VIS spectroscopy resulted in false higher trans-RSV concentrations in conditions under which it was not stable (alkaline pH, light, increased temperature who found that *trans*-resveratrol stability was dependent on temperature; when *trans*-resveratrol was heated to over 30°C, its isomerization form changed from *trans*-resveratrol to *cis*-resveratrol. Additionally, as the temperature increased *trans*-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 *trans*-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.[28] Similarly, Yang *et al.* reported that the TPC of beans roasted for 5 min increased compared to raw beans.[29] and Hečimović *et al.* also reported the comparative study of polyphenols and caffeine in different coffee varieties affected by the degree of roasting.[30] 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.

CONCLUSION

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

ACKNOWLEDGEMENT

The authors wish to acknowledge support from the PITTA 2017 grant issued by the Directorate of Research and Community Engagement (DRPM), Universitas Indonesia.

CONFLICT OF INTEREST

All author declared that have no conflict of interest.

ABBREVIATIONS

HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A; **HPLC:** High-performance liquid chromatography; **TPC:** Total phenolic content; **F-C:** Folin–Ciocalteau; SPSS: Statistical package for the social sciences; **ANOVA:** Analysis of variance; **PITTA:** Publikasi Internasional Terindeks untuk Tugas Akhir Mahasiswa; **DRPM:** Direktorat Riset dan Pengabdian kepada Masyarakat.

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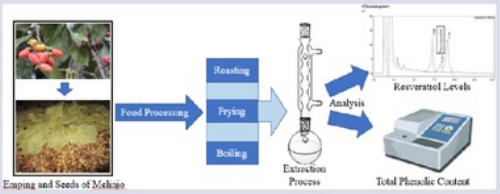
GRAPHICAL ABSTRACT



SUMMARY

- *Trans*-resveratrol is a phenolic compound in the stilbene group present in Melinjo (*Gnetum gnemon* L.) seeds.
- *Trans*-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.
- *Trans*-resveratrol and total phenolic content levels in fried empings decreased was 60%, 68%, and 92% (*trans*-resveratrol) and 41%, 45%, 97% (total phenolic) after 5, 15, and 30 min of boiling.
- The significant changes in *trans*-resveratrol levels and total phenolic content in Melinjo seeds before and after various cooking processes.

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Cite this article: Ahmad I, Andriyani D, Gunawan C, Fauziah ND, Sutriyo S, Mun'im A. Effects of Food Processing on Resveratrol and Total Phenolic Content in Melinjo (*Gnetum gnemon* L.) Seeds. Pharmacog J. 2018;10(6):1096-100.

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