

# NUTRITION



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## Research Article Unlighted Maceration and Ultrasound Technique: The Key to Gaining Stable Trans-Resveratrol from Alternative Sources in Tempeh and Soybean Seed Coat

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### Abstract

**Objective:** The aim of this study was to determine the best extraction conditions to gain stable trans-resveratrol from tempeh and soybean seed coat. **Materials and Methods:** The first stage was to determine trans-resveratrol of tempeh and soybean seed coat during extractions at room temperature, with a mechanical maceration speed up to 180 rpm for 2 h and sonication for 5 min. This method consisted of an orderly extraction with a rotary evaporator at a boiling point of 60°C, rotation of 100 rpm under a vacuum of 556 mBar with an efficiency of 80%, an evaporation enthalpy of 513 J g<sup>-1</sup> and a density of 784 kg m<sup>-13</sup>, followed by N-hexane extraction at a boiling point of 60°C, rotation of 100 rpm under a vacuum of 335 mBar with an efficiency of 80%, an evaporation enthalpy of 368 J g<sup>-1</sup> and a density of 660 kg m<sup>-13</sup> in three cycles for 2 h and 55 min. In this work, the extraction method utilized sonication and maceration under dark conditions. **Results:** Results indicated that acetone is the optimal solvent with a concentration of 70% and a solvent/material ratio of 10/1 (v/w), an extraction time of 175 min, a mechanical maceration of 120 min and sonication for 5 min at 40 kHz at 100 W and 38°C. Trans-resveratrol was obtained and observed by TLC and HPLC. **Conclusion:** These methods signi cantly a ected the stability of trans-resveratrol. The best results used acetone as the solvent with sonication-assisted unlighted maceration extraction.

Key words: Resveratrol, soybean seed coat, tempeh, ultrasound, unlighted maceration

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

#### INTRODUCTION

Recently, consumption of trans-resveratrol-rich foods has become a popular trend that offers health advantages such as neuroprotective effects, notably against neurodegenerative diseases such as Alzheimer's diseases (AD) and, to a minor degree, prevents initial brain upgrade in the newborn<sup>1-4</sup>. Advances in preparation strategies for resveratrol analysis have been disclosed, especially solid-phase extraction (SPE)<sup>5-6</sup>, magnetic hydrophilic/carboxylated multi-walled carbon nanotubes (h/c-MWCNT-MNPs)<sup>7</sup>, stir bar sorptive extraction (SBSE)<sup>6</sup> and liquid-liquid extraction (LLE)<sup>8</sup>, which have shown promising results for detecting resveratrol in red wine.

As reported by Kim *et al.*<sup>9</sup>, Lin *et al.*<sup>10</sup> and Malencic *et al.*<sup>11</sup>, soybeans contain phenolic compounds, such as phenolic acids, which have gained attention because these compounds can be modified by the fermentation process. Therefore, the modification technique to isolate trans-resveratrol in soybean seed coat and tempeh must be developed differently from the method used in red wine.

Since trans-resveratrol is a photosensitive compound and easily isomerizes under ultraviolet irradiation<sup>12</sup>, special attention has been paid toward its isolation. At the same time, an assortment of extraction procedures play roles in the separation and analysis of trans-resveratrol in soybean seed coat and tempeh. Maceration at room temperature<sup>13</sup>, heating reflux extraction<sup>14</sup>, Soxhlet extraction<sup>15</sup> and remarkably microwave-assisted extraction<sup>16</sup> were popularized for transresveratrol extraction. Conventionally, acetone, chloroform, ethyl acetate, ethanol, methanol and some mixed solvents have been applied.

Nonthermal food processing methods, namely, accelerating and comprehensive ultrasound technology, have more estimable use compared to chemical, mechanical, or physical effects on the process<sup>17</sup>. Generally, high-power ultrasound can improve the extraction of intracellular compounds from plant materials. It is assumed that this positive effect of ultrasound in liquid/solid extraction is mainly caused by a phenomenon known as cavitation<sup>18-21</sup>.

Currently, no research has presented the unlighted maceration and ultrasound method for the extraction of trans-resveratrol from tempeh and soybean seed coat as alternative sources. Hence, the goal of this study was to gain stable trans-resveratrol using various novel extraction conditions including the solvent type, solvent concentration, extraction time, extraction modification and solvent/material ratio from tempeh and soybean seed coat.

#### **MATERIALS AND METHODS**

**Experimental site:** This study was conducted at the Laboratory of Chemistry, Department of Chemistry, Faculty of Mathematics and Science, Jakarta State University, Jakarta, Indonesia and the Laboratory of Biomaterial and Biology, (LIPI) Indonesia. Tempeh and soybean seed coat were collected commercially and hand-picked at Sentra Tahu Tempeh Primkopti (Lenteng Agung, South Jakarta, Indonesia).

Materials and research tools: A rotary evaporator (model RV10 of IKA, Werke GmbH & Co.KG, Germany) incorporating a 9.5 L 120 V/60 Hz electric aspirator pump (model VE-11 of Jeio Tech Co. Ltd, Korea) with an RV 10.102 receiving flask of 500 mL capacity and an RV 10.82 evaporation flask of 250 mL capacity were used for extraction. Often, solvents used in pressurized liquid extractions were swirled to disencumber with a rotary shaker (model WIS-30 of WiseCube, Witeg, Germany). To degas the samples, a Digital Ultrasonic Cleaner (model WUC-DO3H) sonicated by a Multi-Frequency Sonication Body (Witeg, Germany) was applied. Extracts were then lyophilized using a FreeZone 1 L Benchtop Freeze Dry System (Catalog #7740030, Labconco, Kansas City Missouri, USA). In the pre-extraction process, the extracts were dried using an oven (model BCL-OF-11E, Lab Companion, Jeio Tech, Inc., Korea) and milled using a food processor (BL-101 PL, Miyako, Indonesia). The extract was stored at -30°C in a BioMedical Freezer (Model LBF-4010U, Daihan Labtechco., LTD, Korea).

Chromatographic analyses were performed in a Shimadzu HPLC system (model RF-10AXL, Shimadzu Corporation, Horiyamashita Hadano Kanagawa, Japan), including LC-20AB prominence pumps, a CTO-20AC prominence column oven, SIL-20AC prominence auto sampler, SPD-20 AV prominence UV/VIS detector, RF-10Axl fluorescence detector, CBM-20A (Lite) controller, DGU-20A5 prominence degasser and computer integrated LC system software using a CAPCELL PAK C18 UG120 S5 column (SHISEIDO CO., LTD, Japan) of 4.6 mml. D×250 mm. Accessory TLC silica gel 60 F 25 20×20 cm glass plates (Merck Millipore) were also used and were visualized using a TLC box viewer 022.9160 CAMAG<sup>®</sup> UV Lamp 4 dual wavelength 254/366 nm, 2×8 W.

The trans-resveratrol standard was obtained from Sigma Chemical Co. N-Hexane (95%, 2508-25LGL) applied as support material was provided by AJAX Fine Chem Pty. Ltd (New Zealand). For the extraction solvents, acetone of PT MULTI CHEMICALINDO was employed. The TLC eluent chloroform (99.8%) was supplied by Global Chemie, ethyl acetate (99.8%) was supplied by Bratachem and formic acid (85%) was supplied by Bratachem. The sample for TLC was diluted in methanol from AJAX Fine Chem Pty. Ltd (New Zealand). The solvents employed were Aqua Pro Injection and Acetonitrile (1.00030.2500, HPLC grade) from Merck.

#### **Research procedure**

#### Preparation of the standard solution of trans-resveratrol:

The trans-resveratrol powder was dissolved in methanol (5 mg  $L^{-1}$ ) and the solution was protected from light. For stability assays, a standard of the solution was made freshly and added directly onto the TLC plate and into the HPLC vial.

**Sample preparation:** Soybean seed coat was dried in an oven for 5 h. Tempeh and dried soybean seed coat (198 g) were milled separately using a food processor. The milled sample was macerated in 1980 mL of acetone for 180 min. The suspensions were maintained on a rotary shaker at 180 rpm for 120 min. The suspensions were then sonicated for 5 min at a frequency of 40 kHz in HF Peak in a 100 W Digital Ultrasonic Cleaner. The collected fractions were filtered through Whatman No. 4 lter paper supported by a porcelain funnel and an electric aspirator pump and were then stored at room temperature overnight.

**Extraction conditions:** The extractions were performed by employing acetone as the solvent in three cycles for 4 h and 6 min at a boiling point of 60°C and a rotation of 100 rpm under a vacuum of 556 mBar with an efficiency of 80%, an evaporation enthalpy of 513 J g<sup>-1</sup> and a density of 784 kg m<sup>-1</sup><sup>-3</sup>. Then, the extraction/elution stage was performed by employing N-hexane at a boiling point of 60°C and a rotation of 100 rpm under a vacuum of 335 mBar with an efficiency of 80%, an evaporation enthalpy of 368 J g<sup>-1</sup>, a density of 660 kg m<sup>-1 3</sup> and in this case, in three cycles for 2 h 55 min.

The extract was kept in a brown bottle and stored at -30°C in a BioMedical Freezer for 48 h. The pale yellow crystal was lyophilized until it existed as a stable weight (approximately 80% of the original weight). The sample was then kept at 4°C until chromatographic analysis. In this analysis, approximately 5 mg of sample in methanol was used.

**Experimental design:** The investigated pale yellow crystal samples were applied by using microcapillaries to the thin-layer chromatographic plates, 3 samples per plate. Samples were spotted onto the plates at a width of 10 mm from each other as well as from the left and the right edge

of the plate and 10 mm above the lower edge. The chromatograms were developed in vertical chromatographic chambers to a width of 7 cm by using chloroform-ethyl acetate-formic acid (2.5:1:01, v/v) as the eluent and visualized in a TLC plate viewing box at a wavelength  $\lambda = 254$  nm. TLC analyses were repeated three times (n = 3) for each investigated sample.

The extracts were filtered through nylon filters of 0.45  $\mu$ m pore size. The mobile phase used solvent A (Aqua Pro Injection) and solvent B (Acetonitrile) (60:40; v: v). The flow rate was 1 mL min<sup>-1</sup> with a linear gradient elution from 10% solvent B and 90% solvent A within 18 min to 85% solvent B and 15% solvent A within 5 min. The UV detector was adjusted at  $\lambda = 306$  nm.

**Parameters measured:** The data collected during this study were determined by TLC and HPLC. The results were based on a standard curve obtained with standard trans-resveratrol. TLC data were expressed as RF values of 0.64 and HPLC data were expressed as the trans-resveratrol peak appearing after an elution time of 14 min for tempeh and 11 min for soybean seed coat.

Statistical analysis: The data were not analyzed statistically.

#### **RESULTS AND DISCUSSION**

Various organic solvents can be used to analyze transresveratrol. One such analysis was performed on grapevine cane extracts using methanol, which is a better solvent than acetone. Acetone produced the lowest percentage value of trans-resveratrol, while the highest percentage of the extract was yielded by methanol. Extracting cut grape canes at laboratory temperature in methanol led to much higher concentrations of trans-resveratrol compared to acetone<sup>22</sup>. Different analyses were employed with optimum conditions of the independent variables. These variables affected the phenolic content extraction efficiency of soybean seeds (Glycine max L.) that used aqueous acetone 70% as the solvent, a 1:6 soybean to solvent ratio, 3 extraction cycles, 3 h of extraction time and a temperature of  $40 \,^\circ C^{23}$ .

Gonzalez-Barrio *et al.*<sup>24</sup> reported that the optimum conditions for grape juice maceration were 2 h at 45°C with 0.2%  $Na_2S_2O_5$  using ultraviolet C (UVC) irradiation, which significantly increased the phenolic (resveratrol) concentration without affecting the sensory properties of the juice. Gambuti *et al.*<sup>25</sup> suggested that 12 days of maceration led to the maximum extraction of trans-resveratrol from Aglianico

Table 1: Review of the literature on ultrasound-assisted extraction of resveratrol

Raw material	Ultrasound-assisted		
	extraction duration	Solvent	References
Cookies and jams	10 min	90% methanol in water	Guaman-Balcazar <i>et al.</i> 40
Arachis repens handro	20 min, 120 rpm	90% MeOH aq	Garcia <i>et al.</i> 41
The roots of polygonum cuspidatum	20 min, 150 W	50% ethanol	Chen <i>et al.</i> <sup>42</sup>

and Piedirosso wines. Furthermore, other studies have established that soybean flour that was fermented by the microbiota present on the seed by A. oryzae, R. oryzae or B. subtilis was identified as stilbene trans-resveratrol-3-O-glucoside in low concentrations. In that study, Duenas *et al.*<sup>26</sup> centrifuged the extracts at 3,000 × g/5175 rpm for 10 min at 5°C and evaporated to dryness at reduced pressure at 30°C. Another analysis was performed on soybeans fermented with the microbiota present on the seed; these samples were sonicated for 20 min and the results showed that one stilbene trans-resveratrol-3-O-glucoside was identified at a low concentration<sup>26</sup>.

Choosing appropriate organic solvents to isolate trans-resveratrol in soybean seed coat and tempeh is required. For this study, an acetone concentration of 70% (v/v) and a solvent ratio of 1:10 (w/v) result in the maximum extraction of trans-resveratrol from tempeh, an Indonesian soybean fermented food and soybean seed coat.

Surprisingly, few reports have attempted isolation of trans-resveratrol from soybean seed coat and tempeh, both of which are Indonesian food sources. Studies in this area address findings in this research, which confirm that trans-resveratrol in soybean seed coat and tempeh was obtained from the modification of maceration between shakers with a speed of 180 rpm for and 2 h and 15 min. In contrast, an isoflavone-enriched tempeh in the form of a granular fermented soybean-based food was prepared by adding soybean germ that contained a large amount of isoflavone, while the major isoflavones in tempeh are genistein and daidzein<sup>27</sup>.

In general, resveratrol synthesis decreased during the fermentation process. However, the decrease in resveratrol suggests that the consecutive arrangement of ultrasound (5 min, 40 kHz, 100 W, 38°C) allows for the solvent to remove the small amount of phenol compounds present. As a result, resveratrol was formed and accumulated in tempeh and soybean seed coat, which was validated by HPLC analysis.

Trans-resveratrol is a phenolic compound whose extraction methods gained much attention as this compound was indicated to be associated with fungus and disease resistance. The solvent and the method of extraction determine the extraction yield<sup>28</sup>. Chemical modification of the

compounds of interest could then occur upon complete extraction <sup>29</sup>. Likewise, the complexity of phenolic compounds and their interaction with other bioactive compounds presented in the sample depend on the modification of the extraction method. These findings, which include the most favorable solvents for extraction, can influence the rate of extraction and the quality of extracted bioactive phenolic compounds<sup>30-32</sup>. According to Silva *et al.*<sup>33</sup>, the method for each particular phenolic source must be ideally designed and optimized<sup>33</sup>. These results show that when a plant material and solvent for extraction are placed in a distortion field, it is necessary to employ physical rotation of the molecular dipoles, which leads to the rapid formation of a solvent and sample matrix<sup>34</sup>.

The application of ultrasound can be forcefully employed to enhance the extraction rate by expanding the mass transfer rates and achievable rupture of the cell wall considering the structure of active superior microcavities to produce yields with decreased extraction time and to diminish solvent consumption<sup>35</sup>. A large number of energies, such as heat, pressure and mechanical shear, were discharged by the formation and collapse of microscopic bubbles during ultrasonic irradiation<sup>36</sup>. Ultrasound resulted in the disruption of cell walls, particle size reduction and enhanced mass transfer across cell membranes accomplished by mechanical and thermal treatment<sup>37-38</sup>. Acceleration of current diffusion and internal diffusion induced the collapse of cavitation bubbles, which generated microturbulence, high-velocity interparticle collisions and perturbation in microporous particles of the plant materials<sup>35,37-39</sup>.

Notably, besides the importance of discovering resveratrol for use as a drug, there is also a concern regarding the efficiency of the current extraction methodologies, which means that ultrasound-assisted dynamic maceration has gained increased interest for finding new resveratrol sources obtained from fermented food and natural products. Thus, resveratrol, in addition to the ultrasound-assisted extraction described above, has been the subject of study for efficient extraction methodologies. Table 1 presents a review of published studies reporting optimal ultrasound extraction conditions, showing that none of the published studies were successful in fermented food.

#### SIGNIFICANCE STATEMENT

This study has discovered that sonication-assisted unlighted maceration extraction is the best extraction method for tempeh and soybean seed coat. This study will help researchers examine the critical area of gaining stable trans-resveratrol during extraction.

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