Steaming Process Does Not Affect The Antioxidant Activities of Tempeh Ethanol Extract

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Abstract. Tempeh is an Indonesian fermented traditional food made from soybeans. It exhibits antioxidant activities mainly due to phytochemicals known as isoflavones. Since tempeh is mainly consumed cooked, this study investigated the influences of steaming process at three different times (10 min, 30 min and 60 min) on the antioxidant activities of tempeh. While a subtle decrease in antioxidant activities was observed in tempeh aqueous extracts, tempeh ethanol extracts did not differ in antioxidant activities. The results suggested that steaming process, even when applied for an hour, caused very minimal decrease in tempeh antioxidant activities. Such a decrease was particularly observed in the water-soluble fraction but not in the ethanol-soluble fraction. Therefore, steaming could be opted as a good cooking method for tempeh to keep its antioxidant-related health benefits.

Keywords : tempeh, soybean, isoflavone, steaming, antioxidant

1. Introduction
Tempeh is a traditional soyfood indigenous to Indonesia whose production involves fermentation by Rhizopus oligosporus. It is commonly found at traditional markets and consumed by Indonesian people as a protein source with high nutritional qualities. During the fermentation, the molds degrade carbohydrates, fats and proteins in the soybeans, resulting in higher bioavailability of such nutrients in tempeh compared to soybeans [1-2]. Tempeh is also believed to provide health benefits to humans, thus making them a potential functional food to develop [3-4].

Several studies have reported an increase in tempeh antioxidant activities during fermentation [5-7]. Indeed, tempeh is rich in bioactive compounds exerting antioxidant activities called isoflavones that are present in three forms: daidzein, genistein and glycitein [8]. In soybeans, isoflavones are mostly present in conjugated forms with sugar molecules (known as glucosides). During fermentation, enzyme beta-glucosidase secreted by R. oligosporus hydrolyzes isoflavone glucosides, leading to the formation of free isoflavones (known as aglycones) with higher bioavailability and antioxidant activities compared to isoflavone glucosides [9-10]. Isoflavones have been linked to the prevention of diseases, including cancer, hypercholesterolemia, atherosclerosis, cardiovascular disease, osteoporosis and relief of menopausal symptoms in certain women [11].

Tempeh is commonly consumed cooked. Exposure to heat during cooking might have detrimental effects towards isoflavones in tempeh. Until now, very few studies have been conducted to study the influence of heat on antioxidant activities in tempeh. Most studies focused on how fermentation time or additional treatments during fermentation might influence the antioxidant activities of raw tempeh. Therefore, this study aimed to investigate whether antioxidant activities of tempeh decrease when tempeh is exposed to steam for three different durations: 10 min, 30 min and 60 min. The results
obtained from this study would give an insight into the sensitivity of antioxidants in tempeh towards heat generated from steaming process and, eventually, provide suggestions with regard to the optimal means to cook tempeh in order to not lose its antioxidant activities.

2. Materials and Methods

2.1. Sample Preparation
Tempeh fermentation was done as previously described [12] with some modifications. Briefly, soybeans (var Anjasmoro, harvest age 85 days, produced by UD Sumber Makmur, Nganjuk, East Java, Indonesia) were inoculated with starter (brand Unggul, produced by PD Sukma Jaya, Tegal, Central Java, Indonesia, 5g/kg soybean) and left fermented at room temperature (25°C) for 36 h to produce raw tempeh. Tempeh was cut into cubes (2x2x2 cm3) and steamed by using a rice cooker whose container was filled with distilled water at half volume. The water was boiling when the tempeh cubes were put into the rice cooker and the cooking time started to be counted. Three cooking durations were tested: 10 min, 30 min and 60 min. After the cooking durations were reached, tempeh cubes were taken out from the rice cooker and left cooled. To produce tempeh aqueous and ethanol extracts, raw tempeh and/or steamed tempeh were mixed with distilled water or ethanol 95% with a ratio of 1:3 (w/v) by using a blender, resulting in puree. The puree was then filtered by using a cheese cloth to separate the pulp from the filtrate. The filtrate was collected and kept in a freezer (-20°C) for further analyses. Soybean extracts were prepared using the same method as tempeh extracts.

2.2. Total Phenols, Total Phenols and Antioxidant Activities
The three analyses were done by spectrophotometry. Total phenols and total flavonoids, analyzed as previously described [13-14] with some modifications, were expressed as gallic acid equivalents (GAE) and quercetin equivalents (QE) respectively. The analysis of antioxidant activities was done based on a,a-diphenyl-b-pticryl-hydrazyl (DPPH) radical scavenging analysis as previously described [15] with some modifications. Antioxidant activities were expressed as percentage of free radical inhibition obtained by using the following equation: [(Acont-Atest) / Acont]x100%, where Acont is the absorbance of DPPH solution and Atest is the absorbance of DPPH solution mixed with tested extracts.

2.3. Statistical Analysis
Data (n=3) were analyzed using software Systat 10 software for Windows. All data were reported as mean±SD. Data were divided into four groups: soybean (water), soybean (ethanol), tempeh (water) and tempeh (ethanol), as presented in Figure 1A-C. Data within each group were analyzed by one-way ANOVA followed by Dunnett’s test in case of significant differences (p<0.05). Analysis of two data from two different groups was performed by student’s t-test (p<0.05).

3. Results and Discussion
As presented in Figure 1A-C, tempeh extracts exhibited higher total phenols, total flavonoids and free radical inhibitory activities compared to soybean extracts, regardless of the solvent used to extract tempeh. Ethanol fractions also tended to show greater antioxidant profile and activities compared to aqueous fractions. Interestingly, tempeh ethanol extracts did not show any statistically significant reduction in either total phenols, total flavonoids or free radical inhibition (p≥0.05) due to steaming process. In contrast, tempeh aqueous extracts seemed to be more susceptible to steaming process than tempeh ethanol extracts, especially at t=60 min where the decrease in total phenols, total flavonoids and antioxidant activities were statistically different (p<0.05). Despite exhibiting relatively much lower values compared to tempeh extracts, soybean extracts (water and ethanol) displayed a decline in total phenols and total flavonoids particularly after being steamed for 60 min.

In this study, we extracted soybeans and tempeh in distilled water and ethanol. We had two arguments to justify our choice of solvents. Firstly, the major compounds exerting antioxidant activities in soybeans and tempeh are very likely to be isoflavones and other phenolic compounds. Isoflavones are considered water-soluble, but they have a greater solubility in slightly less polar
solvents such as methanol or ethanol [16]. Isoflavone glycosides are highly soluble in water while isoflavone aglycones have a very low solubility in water (about 1,000-10,000 times as low as isoflavone glycosides) [17]. Secondly, water extract and ethanol extract of tempeh have been previously shown to give greater antioxidant activities compared to tempeh extract in nonpolar solvents such as hexane, petroleum ether or ether [18].

From Figure 1A-C, we can deduce that ethanol is a better solvent for extracting isoflavones in soybeans and tempeh compared to distilled water. Despite possessing oxygen atoms allowing the creation of hydrogen bonds with polar molecules, the structure isoflavones also contains benzene rings that contribute to their nonpolarity. This might explain why isoflavones are more soluble in slightly nonpolar solvents like ethanol compared to purely polar solvent like water. Recently, a study comparing methods for extraction of isoflavones reported that nonacidified 80% aqueous methanol is the preferred extraction solvent for isoflavones since it provides the best yields with very little transformations of the naturally occurring forms of isoflavones present in the food [19]. However, one should take precautions when handling methanol in the laboratory since it is extremely toxic to humans if ingested or if vapors are inhaled.

Regardless of the solvent used for extraction, tempeh extracts exhibited higher antioxidant profile and activities compared to soybean extracts (Figure 1A-C). This finding is in accordance with previous studies revealing that antioxidant activities of soybeans increased during tempeh fermentation [6,18,20-22] and such an increase was accompanied by increasing isoflavone aglycone/glycoside ratio [23]. Isoflavone aglycones have been reported to exert stronger antioxidant activities in LDL oxidation assay compared to isoflavone glycosides [10].

Several studies have reported that isoflavones are sensitive towards heat. Isoflavones are least stable in an environment with elevated pH and temperature [24]. Autoclaving soymilk at 121°C for 15 min led to a loss of 20% of total isoflavones [25]. Upon exposure to excessive heat, isoflavone glycosidases tend to be hydrolyzed, thus resulting in isoflavone aglycones and free sugar molecules. When the heating continues for a relatively long time, isoflavone aglycones can be degraded and lose their antioxidant activities [26-29]. It is therefore plausible to deduce that cooking process could harm isoflavones in soybeans and tempeh, particularly when excessive temperature is used in such a process.

In this study, steaming process did not affect the antioxidant profile and activities of tempeh ethanol extracts but reduced the ones of tempeh aqueous extracts only after 1 h of cooking (Figure 1A-C). We hypothesized that the combination of temperature and time in our study was less excessive compared to the conditions imposed in the previous studies [26-29]. However, it is noteworthy that steaming process of food takes usually 5-15 minutes and at this time, there was no reduction in total phenols, total flavonoids or antioxidant activities observed in both tempeh aqueous and ethanol extracts. Interestingly, in accordance with our study, an experiment investigating the effects of different cooking methods on isoflavone content in soyfoods reported that soft tofu steamed with soy sauce at 60°C for 10 min exhibited similar isoflavone content with uncooked soft tofu. Other cooking processes comprising boiling or frying caused a reduction in isoflavones up to 37.56% [30]. For instance, deep frying battered tempeh for 30 min caused a loss in isoflavone content of 45% compared to raw tempeh [31].
Figure 1. Total phenols (A), total flavonoids (B) and free radical inhibition (C) of water and ethanol extracts of soybeans and tempeh (steamed or not). Data (n=3) are reported as mean±SD. *) Significant difference with « unsteamed » in the same group ((p<0.05). #) Significant difference between water extract and
ethanol extract of the same sample (soybeans/tempeh) undergoing the same treatment (unsteamed/steamed) (p<0.05). $) Significant difference between soybeans and tempeh undergoing the same treatment (unsteamed/steamed) extracted using the same solvent (water/ethanol) (p<0.05).

4. Conclusions
Steaming process is very little invasive towards isoflavones present in tempeh. Steaming tempeh at 100°C for 60 min caused very little reduction in tempeh antioxidant activities while no reduction was observed when tempeh was steamed for 10 min or 30 min. Tempeh ethanol extracts were more resistant towards detrimental effects compared to tempeh aqueous extract. We therefore suggest to opt for steaming over other more invasive methods (boiling or frying) when it comes to cooking tempeh in order to minimize isoflavone loss and to obtain the maximum health benefits offered by tempeh.

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