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# The impact of pressure boiling of dry peeled soybeans on the isoflavone content of tempeh

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## Abstract

Isoflavones are bioactive compounds which are components of flavonoid compounds. Soybeans, the main ingredient of tempeh, contain these isoflavones that act as antioxidants. During the cooking process of tempeh, isoflavones become very unstable due to heat. Therefore, the boiling stage is a very important concern in the tempeh-making process. In the conventional tempeh-making method, the soybeans are boiled twice, with each boiling time carried out between 2 and 3 h after the water boils, based on a survey from tempeh SMEs. This conventional boiling time increases fuel costs, especially when processing large volumes of soybeans. This research aimed to find a boiling method with a shorter cooking time and evaluate its impact on the isoflavone content of the tempeh produced. The experiment tested boiling times of 10, 20, and 30 min for dried peeled soybeans using pressure boiling. Variables observed include the isoflavone profile and weight in the tempeh. The results from the LC-MS/MS test showed six components of tempeh isoflavones in the control treatment and after 10 min of boiling. These components were daidzein, genistein, daidzin, genistin, glycitein, and glycitin. Glycitein did not appear in the 20 and 30 min of boiling treatments, leaving only five isoflavone components in those treatments. The weight of the isoflavones for each boiling time was 180.312 mg/100 g for 10 min treatment, 211.228 mg/100 g for 20 min, 118.703 mg/100 g for 30 min, and 407.247 mg/100 g in the control treatment. The data showed that using pressure boiling in tempeh production to boil soybeans would shorten the time, but it would also accelerate the loss of isoflavone weight in the tempeh.

## 1. Introduction

Boiling is one of the process stages in tempeh production, which determines the success of the final tempeh result, because it is related to the growth of the fungus in the fermentation process. In the making of tempeh, before boiling, the steps of soaking and peeling the epidermis of the soybean seeds are carried out in wet conditions, leading to the need for a longer time and more water. The results of a research survey conducted at the home industries in Sanan village, Malang, depicted that on average, craftsmen boiled soybeans between 2 and 3 h after water boiled for soybean raw material volumes of 50 to 100 kg with 2 boiling times. Research conducted by Yudiono *et al.* (2021) utilized the conventional method of boiling soybeans, which was also carried out for 2 times, each taking 30 to 60 min after the water boiled for 300 g of sample. This was

deemed by the researchers to be inefficient; a breakthrough was needed. In line with the opinion of Kinyanjui *et al.* (2014) that boiling soybeans for tempeh making requires excessive time and fuel, and ultimately causes production inefficiencies. Meanwhile, according to (Navajas-Porras *et al.*, 2020; Zhang *et al.*, 2023), the seed cooking method is reported to increase the percentage of leached solids, thereby reducing the content of chemical compounds, especially bioactive compounds, in the material. An effort to shorten the cooking time in making tempeh is by soaking the soybeans before the boiling stage. Even though the soaking process has been proven to shorten the boiling time, it has not been able to shorten the total time required for making tempeh using the conventional method of around 4-5 days, because the soaking process alone usually takes around 24 h. Kinyanjui *et al.* (2014) stated that in order to reduce the soaking time for grains,

it can be done by stripping the seed coat before soaking, which is known as dry peeling. The dry peeling method means that after the soybeans are sorted, they are immediately peeled using a peeling machine, without going through the soaking or boiling process. Peeling soybeans occurs because there is friction between the soybeans and the components of the equipment. According to Ansar and Abdullah (2022), the components of this peeling machine include two circular grinding stones, where both rotate or one rotates while the other remains static; a rotating screw with a main screw and a screw wall; and two rubber rollers arranged in parallel, of the same size, which rotate in opposite directions but with different angular velocities. According to (Kusumawati *et al.*, 2020), dry peeling (skin breaking) has been proven to shorten the time by 2 h, save clean water because no soaking is required, and save fuel. Another advantage of the dry peeling process is that it can be done outside the production stage independently, without having to wait for the completion of the tempeh production process. Therefore, this method has a great potential for the development of large-scale modern industry and exports, and is robust against soybean price fluctuations by utilizing futures trading.

The innovation in this research is boiling dry peeled soybeans using temperatures above the boiling point of water and pressure above the atmospheric environment, with the aim of shortening boiling time and preventing damage to nutrients and phytochemical compounds in soybeans. This method is known as Subcritical Water (SCW). According to King *et al.* (2009), subcritical water is liquid/water that is heated to a temperature between the boiling point (100°C) and below the critical water temperature (374°C). Ju and Howard (2005) stated that subcritical water extraction is carried out at temperatures above 100°C but below the supercritical water temperature (374°C), with sufficient pressure to keep the water in the liquid phase. Likewise, Celabor (2016) stated that boiling/cooking treatment with water/liquids under pressure at temperatures above the normal boiling point (100°C or 212°F) is known as "subcritical water" or "pressurized hot water".

The use of pressure above atmospheric levels is beneficial not only for sterilizing microbes but has also been studied in relation to food ingredients, such as its effect on the quality attributes of aquatic products (Anggo *et al.*, 2018; Crotova *et al.*, 2020); its effect on food safety and quality (Considine *et al.*, 2008); and its effect on the nutrients and phytochemical compounds of potatoes (Tsirikika *et al.*, 2019).

However, its application to soybeans has never been examined, especially as a processing stage for tempeh production. The application of the SCW method is very

promising to replace the conventional soybean boiling stage, in particular for retaining bioactive compounds and increasing production efficiency, which saves fuel, clean water and processing time. The results of preliminary experiments showed that boiling soybeans in an autoclave at a pressure above 1 atm with heating times of 5 to 10 min produced softening of the texture and expansion of soybean seeds, which is advantageous when used for tempeh. Texture softening at high temperature and pressure results in starch gelatinization, protein denaturation, and texture softening (Wang and Daun, 2005), whereas according to Considine *et al.* (2008), it will cause membrane disruption and loss of turgor pressure in order that cell separation will occur. An autoclave is a pressure heating device used for sterilization, while in the household, a cooking device whose working mechanism resembles an autoclave is known as a Presto and is usually used to speed up the softening of the ingredients cooked. The autoclave works effectively if water vapor can penetrate all parts of the material being heated; therefore, the heated material should not fill all space (Adji *et al.*, 2007)

Although the results of temperature and pressure boiling of dried peeled soybeans at (temperature >100°C, pressure >1 atm) in preliminary research are much more efficient than conventional methods in making tempeh, it is necessary to study the impact on the chemical content, especially phytochemical/bioactive compounds such as flavonoids, mainly isoflavones. In addition, some water-soluble phytochemical compounds can be leached due to the penetration of water into the ingredients during pressure cooking (Ng *et al.*, 2011). Veda *et al.*'s research (2010) examined cooking at a pressure of 15 psi for 10 min; it caused a decrease in carotenoid content in spinach, in drumstick leaves, in coriander leaves and Chinese mustards (Bembem *et al.*, 2014). On the other hand, other researchers reported that pressure cooking increased the total phenolic concentration in Chinese mustard greens (Bembem *et al.*, 2014) and drumstick leaves. (Thummakomma *et al.*, 2018). The application of pressure in the cooking process is likely to cause softening of the vegetable matrix, thereby increasing the extraction capacity, releasing phenolic compounds from the pectin or cellulose network, forming secondary plant metabolites, inactivating oxidative enzymes (e.g. polyphenol oxidase), and releasing active aglycones from flavonoid conjugates during heating (Jiménez-Monreal *et al.*, 2009; Bembem *et al.*, 2014; Thummakomma *et al.*, 2018).

Teixeira-Guedesa *et al.* (2019) claimed that cooking under pressure conditions will cause the phenolic acid and flavonoid content in bean seeds to decrease when the cooking water is drained, whereas the total phenolics and

total flavonoids in the boiled water increase. Fathinatullabibah *et al.* (2014) asserted that flavonoids are stable at a temperature of 70°C. Furthermore, it is revealed that flavonoids are a class of compounds that are not heat-resistant and are easily oxidized at high temperatures (Rompas *et al.*, 2012). Flavonoids are semi-polar compounds because they have several hydroxyl groups or a sugar (in the form of glycosides), so they dissolve in semi-polar/polar solvents such as ethanol, methanol and water, especially water in subcritical water conditions (Yudiono, 2011). Isoflavones are included in the flavonoid group, which are polyphenolic compounds. Flavonoids have 15 carbon atoms arranged in a C6-C3-C6 configuration, meaning that the carbon skeleton consists of two C6 groups (substituted benzene rings) connected by a three-carbon aliphatic chain (Wang *et al.*, 2018). Isoflavones consist of 2 benzene rings (A and B) and bind to a heterocyclic pyran C ring, where the B ring is bound by carbon number 3. Isoflavones are found in many soybeans with the constituent components being genistein, daidzein, glycitein, coumestrol and pluerarin, which act as antioxidants (Urasopon *et al.*, 2008).

## 2. Materials and methods

### 2.1 Equipment and materials

#### 2.1.1 Equipment

The equipment used includes a Mass Spectrophotometer (TSQ Quantum Access Max), LC (Accella 1250) made in the United States, an ultrasonic cleaner, micropipette, analytical balance, and vials.

#### 2.1.2 Materials

The raw materials used are dried local soybeans of the Anjasmoro breed, which were harvested from the Malang branch of the Research Institute of Various Legumes and Bulbs (Balitkabi). The chemicals used were methanol and formic acid (PA) obtained from Merck KGaA, Germany.

### 2.2 Pressure boiling preparation of soybeans

Dry peeled soybeans, which were cleaned and split into two parts, were put into a beaker containing water (1:1 g/mL). Next, it was boiled in an autoclave with a boiling time of 10, 20, and 30 min after the water boiled.

### 2.3 Preparation of tempeh

After boiling the soybeans in an autoclave, the soaking was done in 0.25% citric acid or at a pH of 4-4.5. After that, the seeds were drained and then evenly placed on the "tampah" (tray made of woven bamboo) to undergo the cooling process until the temperature was below 40°C. The next process was fermentation, in

which the soybean seeds were dried from boiling water and then mixed with 0.2% of tempeh yeast (*Rhizopus sp.*) by sprinkling the yeast evenly over the soybeans. The soybeans were stirred until the yeast was evenly mixed, and then the whole mixture was wrapped in a 250 g plastic bag which had 15 holes on its top and bottom sides, and 3 holes each on its right and left sides. The last stage was the curing/fermentation process for 48 hrs at room temperature (28-30°C). Prior to analysis, the fresh tempeh was stored at -20°C. In this study, the procedure for making tempeh was different from the conventional method, except for the control (the control followed the procedure by Yudiono *et al.*, 2021).

### 2.4 Preparation of compound tests with LC-MS/MS

Approximately 10 g of tempeh were weighed, then 25 mL of methanol solution was added. Sonification was carried out for 30 min, followed by centrifugation, which was carried out at a speed of 10,000 rpm for 10 min and 5 mL of the filtrate was taken. Afterwards, 25 mL of hexane was added, then sonicated again for 10 min. Finally, the bottom layer was taken and filtered with a size of 0.2 µm and ready for testing with LC- MS/MS.

### 2.5 LC-MS/MS operating conditions

The column used was with Phenomenex specifications (50 mm × 2.1 mm × 1.7 µm). UHPLC brand ACCELLA type 1250 made by Thermo Scientific, which consists of a vacuum degasser, quaternary pump, and thermostatic autosampler, was controlled by a personal computer using the x-calibur 2.1 program. Mobile phase A consisted of 0.1% formic acid in aquabidest, while phase B consisted of 0.1% formic acid in methanol. A linear gradient at a speed of 300 µL/min was performed with the following mobile phase settings: 0-0.6 min 20%B, 0.6-4 min 85%B, 4-5 min 80%B, 5-7.5 min 20%B. The column was controlled at 30°C, and the autosampler compartment was set at 16°C. The use of the TSQ QUANTUM ACCESS MAX mass spectrometer from Thermo Finnigan with an ESI (Electrospray Ionization) ionization source was controlled by TSQ Tune software. The ESI ionization conditions were as follows: spray voltage of 3 kV; evaporation temperature at 300°C; capillary temperature at 300°C; nitrogen as sheath gas pressure at 40 psi, and Aux gas pressure at 10 psi with argon gas. Ionization was carried out on a positive charge with the SRM (Selected Reaction Monitoring) mode setting.

## 3. Results and discussion

### 3.1 Isoflavone profile

The results of isoflavone content characterization of tempeh, using LC-MS/MS with soybean raw materials

boiled under pressure conditions (autoclave) for durations of 10, 20, and 30 min, are depicted in Figures 1, 2 and 3. The condition without pressure (control) is presented in Figure 4.

In Figures 1a, 2a, 3a, and 4a,  $[M-X]^+$  is  $m/z$  198.50 to 199.50, and molecular ion peaks  $[M]^+$  are  $m/z$  255.000, so these results confirmed that the constituent of isoflavon is Daidzein. In Figures 1b, 2b, 3b, and 4b,  $[M-X]^+$  is  $m/z$  152.50 to 153.50 and molecular ion peaks  $[M]^+$  are  $m/z$  271.000. These results confirmed that the constituent of isoflavon is Genistein. In Figures 1c, 2c, 3c, and 4c,  $[M-X]^+$  is  $m/z$  254.50 to 255.50 and molecular ion peaks  $[M]^+$  is  $m/z$  417.000. These results confirmed that the constituent of isoflavon is Daidzin. In Figures 1d, 2d, 3d, and 4d,  $[M-X]^+$  is  $m/z$  270.50 to 271.50 and molecular ion peaks  $[M]^+$  is  $m/z$  433.000 (Genistin). In Figures 1e and 4e,  $[M-X]^+$  is  $m/z$  269.50 to 270.50, and molecular ion peaks  $[M]^+$  is  $m/z$  285.000, so these results confirmed that the constituent of isoflavon is Glycitein.

However, in the 20- and 30-minute treatments (Figures 2e and 3e), the isoflavone components were unknown/undetectable because the chromatogram peak that appeared was not from the isoflavone component

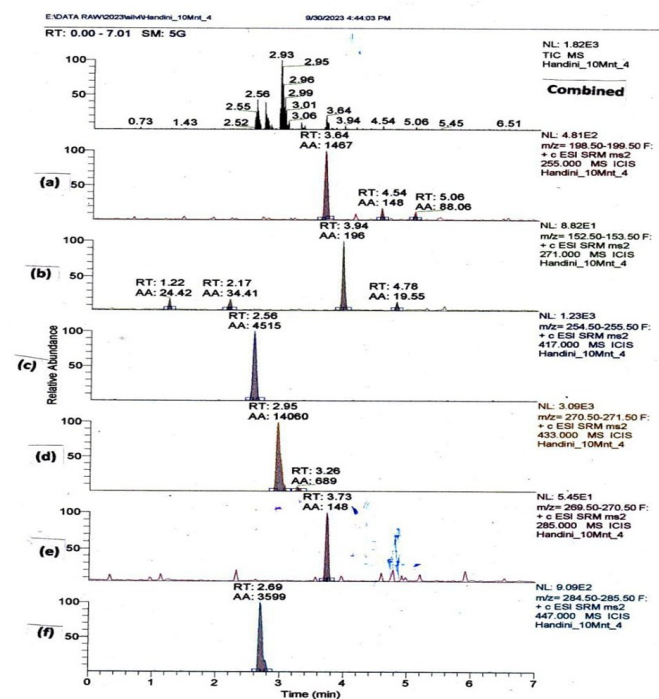


Figure 1. Chromatogram of tempeh isoflavones by pressure boiling dried peeled soybeans for 10 min: (a)  $[M-X]^+$  is  $m/z$  198.50 to 199.50 and molecular ion peaks  $[M]^+$  is  $m/z$  255.000 (Daidzein); (b)  $[M-X]^+$  is  $m/z$  152.50 to 153.50 and molecular ion peaks  $[M]^+$  is  $m/z$  271.000 (Genistein); (c)  $[M-X]^+$  is  $m/z$  254.50 to 255.50 and molecular ion peaks  $[M]^+$  is  $m/z$  417.000 (Daidzin); (d)  $[M-X]^+$  is  $m/z$  270.50 to 271.50 and molecular ion peaks  $[M]^+$  is  $m/z$  433.000 (Genistin); (e)  $[M-X]^+$  is  $m/z$  269.50 to 270.50 and molecular ion peaks  $[M]^+$  is  $m/z$  285.000 (Glycitein); (f)  $[M-X]^+$  is  $m/z$  284.50 to 285.50 and molecular ion peaks  $[M]^+$  is  $m/z$  447.000 (Glycitein).

when viewed from the retention time (RT). In Figures 1f, 2f, 3f, and 4f,  $[M-X]^+$  is  $m/z$  284.50 to 285.50 and molecular ion peaks  $[M]^+$  is  $m/z$  447.000. These results confirmed that the constituent of isoflavon is Glycitein.

### 3.2 Tempeh isoflavone content

The results of chromatogram area and weight of tempeh isoflavone components are presented in Table 1. The results of qualitative observations, obtained by calculating the area of the chromatogram, showed that all treatments of boiling dry peeled soybean raw materials under pressure (autoclave) caused more isoflavone loss compared to the control (boiling wet peeled soybeans without pressure). These results were confirmed by quantitative calculations using standard isoflavone components. In Table 1, it can be seen that the isoflavone component of tempeh in the dry peeled soybean raw material boiling treatment under pressure conditions above 1 atm and temperature above 100°C decreased by more than 50% compared to the control (wet peeled soybeans boiled under pressure and room temperature conditions) despite shorter boiling time. Data taken from a boiling device (autoclave) with boiling durations of 10, 20, and 30 min, at recorded temperatures (109, 123 and

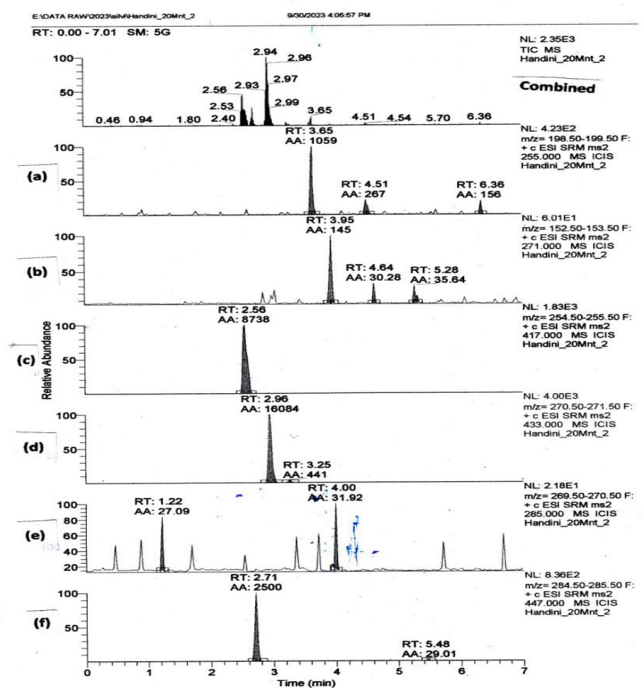


Figure 2. Chromatogram of tempeh isoflavones by pressure boiling dried peeled soybeans for 20 min: (a)  $[M-X]^+$  is  $m/z$  198.50 to 199.50 and molecular ion peaks  $[M]^+$  is  $m/z$  255.000 (Daidzein); (b)  $[M-X]^+$  is  $m/z$  152.50 to 153.50 and molecular ion peaks  $[M]^+$  is  $m/z$  271.000 (Genistein); (c)  $[M-X]^+$  is  $m/z$  254.50 to 255.50 and molecular ion peaks  $[M]^+$  is  $m/z$  417.000 (Daidzin); (d)  $[M-X]^+$  is  $m/z$  270.50 to 271.50 and molecular ion peaks  $[M]^+$  is  $m/z$  433.000 (Genistin); (e) The isoflavone components were unknown/undetectable because the chromatogram peak that appeared was not from the isoflavone component; (f)  $[M-X]^+$  is  $m/z$  284.50 to 285.50 and molecular ion peaks  $[M]^+$  is  $m/z$  447.000 (Glycitein).

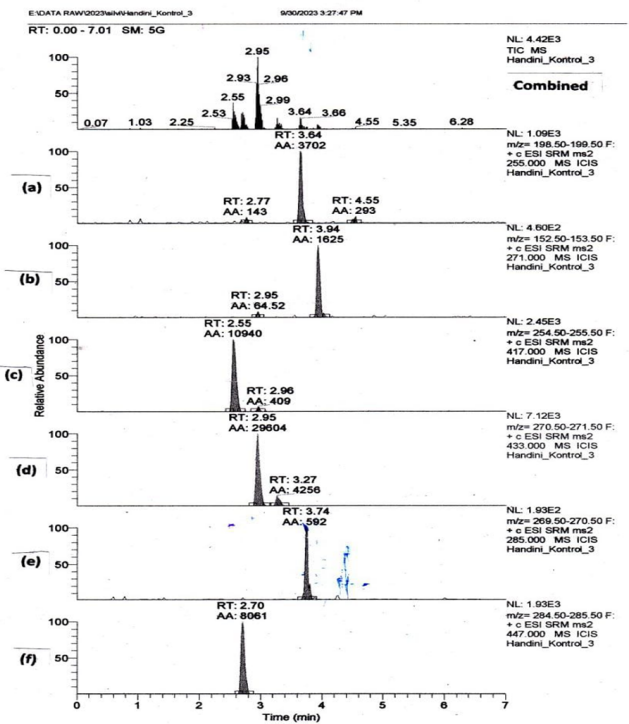
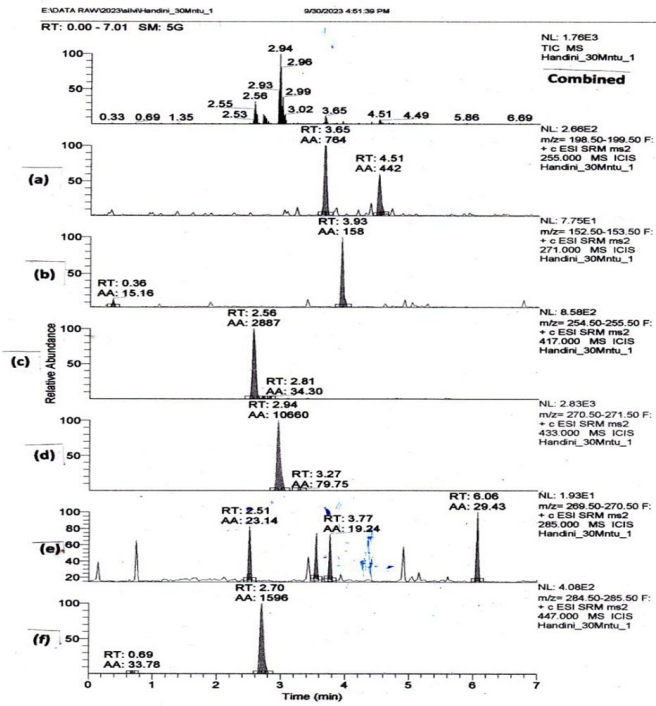


Figure 3. Chromatogram of tempeh isoflavones by pressure boiling dried peeled soybeans for 30 min: (a)  $[M-X]^+$  is  $m/z$  198.50 to 199.50 and molecular ion peaks  $[M]^+$  is  $m/z$  255.000 (Daidzein); (b)  $[M-X]^+$  is  $m/z$  152.50 to 153.50 and molecular ion peaks  $[M]^+$  is  $m/z$  271.000 (Genistein); (c)  $[M-X]^+$  is  $m/z$  254.50 to 255.50 and molecular ion peaks  $[M]^+$  is  $m/z$  417.000 (Daidzin); (d)  $[M-X]^+$  is  $m/z$  270.50 to 271.50 and molecular ion peaks  $[M]^+$  is  $m/z$  433.000 (Genistin); (e) The isoflavone components were unknown/undetectable because the chromatogram peak that appeared was not from the isoflavone component; (f)  $[M-X]^+$  is  $m/z$  284.50 to 285.50 and molecular ion peaks  $[M]^+$  is  $m/z$  447.000 (Glycitin).

Figure 4. Chromatogram of tempeh isoflavones by boiling without pressure on wet peeled soybeans for 30 min (control): (a)  $[M-X]^+$  is  $m/z$  198.50 to 199.50 and molecular ion peaks  $[M]^+$  is  $m/z$  255.000 (Daidzein); (b)  $[M-X]^+$  is  $m/z$  152.50 to 153.50 and molecular ion peaks  $[M]^+$  is  $m/z$  271.000 (Genistein); (c)  $[M-X]^+$  is  $m/z$  254.50 to 255.50 and molecular ion peaks  $[M]^+$  is  $m/z$  417.000 (Daidzin); (d)  $[M-X]^+$  is  $m/z$  270.50 to 271.50 and molecular ion peaks  $[M]^+$  is  $m/z$  433.000 (Genistin); (e)  $[M-X]^+$  is  $m/z$  269.50 to 270.50 and molecular ion peaks  $[M]^+$  is  $m/z$  285.000 (Glycitein); (f)  $[M-X]^+$  is  $m/z$  284.50 to 285.50 and molecular ion peaks  $[M]^+$  is  $m/z$  447.000 (Glycitin).

Table 1. Tempeh isoflavone component levels (genenstein equivalent) based on LC-MS/MS chromatogram.

Tempeh isolavone componen	Boiling dried peeled soybeans in an autoclave						Boil wet peeled soybeans in a pan (30 min)	
	10 min*		20 min**		30 min***		Control****	
	Chr. area/g	weight (mg/100 g)	Chr. area/g	weight (mg/100 g)	Chr. area/g	weight (mg/100 g)	Chr. area/g	weight (mg/100 g)
Daidzein	146	10.953	103	7.747	75	5.556	364	27.581
Genistein	19	1.361	14	0.960	15	1.057	160	12.041
Daidzin	448	33.958	853	64.765	282	21.319	1076	81.736
Genistin	1395	105.997	1570	119.309	1040	79.036	2912	221.381
Glycitein	15	0.999	n.d.	0	n.d.	0	58	4.312
Glycitin	357	27.044	244	18.446	156	11.734	793	60.195
Total		180.312		211.228		118.703		407.247

n.d.: not detected.

The results of recording temperature and pressure in the autoclave are as follows:

\* The boiling time is 10 min after the water boils, the final temperature is reached at 109°C and the absolute pressure is 1.408 atm.

\*\* The boiling time is 20 min after the water boils, the final temperature is reached at 123°C and the absolute pressure is 2.157 atm.

\*\*\* Boiling time is 30 min after the water boils, the final temperature is reached at 131°C and the absolute pressure is 2.701 atm.

\*\*\*\* Boiling under room atmospheric pressure for 30 min after the water boils.

131°C) with absolute pressure (1.408, 2.157, and 2.701 atm). In these conditions, there are changes in the properties of the water, such as a decrease in polarity, in viscosity and in surface tension as a result of an increase in water diffusivity, with the purpose that the dissolution of isoflavone compounds by water will be faster and more effective. Heating water above its boiling point and pressurizing it above 1 atm to create subcritical water increases the mobility of water molecules, enhancing its ability to rapidly penetrate and exit plant tissue, thereby accelerating the dissolution of compounds within the plant matrix (King and Grabiell, 2007; King *et al.*, 2009; Ghoreishi and Shahrestani, 2009). Increasing pressure causes an increase in the density of water; as a result, it will increase the dissolving power of water (Richter *et al.*, 2006). Besides that, at high pressure and high temperature, water behaves like gas and water to such an extent that it has a positive effect on dissolving power (Richter *et al.*, 2006). Dávila *et al.* (2013) reduced water polarity, causing phenolic compounds, including isoflavones, which are relatively less polar, to be dissolved and excreted more easily from plant tissue, even without using organic solvents such as methanol and ethanol. From Table 1, it is also confirmed that there are 3 of the most abundant isoflavone components in tempeh, which are: Daidzin, Genistin and Glycitin, meaning that even though the soybeans have undergone fermentation, the most dominant form of tempeh isoflavones are in the form of aglycones. This can be attributed to the fact that in this research, the process of making tempeh did not go through the soaking stage, as in the conventional method. Liu *et al.* (2023) stated that 99% of isoflavone glycosides (glycones) in raw soybean seeds, during the soaking process and the tempe making process, can be hydrolyzed into isoflavone and glucose aglycones. While Toda *et al.* (2001) reported that one of the important factors in the changes occurring during soaking is the release of isoflavone compounds in free form (aglycones), especially the formation of Factor-II compounds (6,7,4' tri-hydroxy isoflavone). Likewise, the results of research by de Lima *et al.* (2014) claimed that soaking soybeans at a temperature of 55°C for 3 h could increase the isoflavone aglycone content by 6 times. The results of the isoflavone analysis of soybean powder without processing were smaller than those of soaked soybeans (Failisnur *et al.*, 2015). Based on this, making tempeh using a pressure cooker using dried peeled soybeans as raw material is indeed very fast, efficient and economical, but it is necessary to find a solution to hinder the reduction of isoflavones from tempeh products.

#### 4. Conclusion

The profiles of soybean isoflavones produced in the

10 min boiling time and the control treatment contain 6 components, which are: Daidzein, Genistein, Daidzin, Genistin, Glycitein, and Glycitin, while in the 20 and 30 min boiling treatments, they only show 5 components, where the Glycitein component is not detected. The isoflavone content of all treatments is lower than that of the control (boiling without pressure). The impact of boiling soybeans with pressure above 1 atm, especially for tempeh producers, can shorten the boiling time and lead to energy consumption savings.

#### Conflict of interest

The authors declare no conflict of interest.

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#### References

- Adji, D., Zuliyanti and Larashanty, H. (2007). Comparison of the effectiveness of sterilization of 70% alcohol, infrared, autoclave and ozone on the growth of *Bacillus subtilis* bacteria. *Jurnal Sain Veteriner*, 25(1), 18-26. <https://doi.org/10.22146/jsv.275>
- Anggo, A.D., Riyadi, P.H., Rianingsih, L. and Wijayanti, I. (2018). TTSR (High Temperature, Low Pressure) method in processing soft thorn milkfish. *Journal of Food Science and Agricultural Products*, 2(1), 13-24. <https://doi.org/10.26877/Jiphp.V4i2.7516>
- Ansar, A., and Abdullah, S.H. (2022). The technique for peeling the epidermis of soybean seeds using a peeling machine. *Jurnal Ilmiah Abdi Mas TPB Unram*, 4(2), 146-151. <https://doi.org/10.29303/abdimas.v4i2.1061>
- Bembem, K., Sadana, B. and Bains, K. (2014). Effect of domestic cooking methods on the nutritive and antioxidative components of mustard leaves (*Brassica juncea*). *International Journal of Food Agriculture and Veterinary Sciences*, 4(1), 24-31. <https://doi.org/10.5958/2322-454X.2024.00004.4>
- Celabor (2016). Subcritical water. Retrieved on October 25, 2023 from Celebor website: [https://www.celabor.be/site/FCK\\_STOCK/Media/2016-08%20Eau%20sub:critique%20EN.pdf](https://www.celabor.be/site/FCK_STOCK/Media/2016-08%20Eau%20sub:critique%20EN.pdf).
- Considine, K.M., Kelly, A.L., Fitzgerald, G.F., Hill, C. and Sleator, R.D. (2008). High pressure processing—Effect on microbial food safety and food quality. *FEMS Microbiology Letters*, 281(1), 1-9. <https://doi.org/10.1111/j.1574-6968.2008.01084.x>

- Cropotova, J., Mozuraityte, R., Stendal, I.B., Ojha, S., Rustad, T. and Tiwari, B. (2020). Influence of high-pressure processing on quality attributes of haddock and mackerel minces during frozen storage, and fishcakes prepared thereof. *Innovative Food Science and Emerging Technologies*, 59, 102236. <https://doi.org/10.1016/j.ifset.2020.102236>
- Dávila, J.A., Sancho, M.I., Almandoz, M.C. and Blanco, S.E. (2013). Solvent effects on the dissociation constants of hydroxyflavones in organic–water mixtures: Determination of the thermodynamic pKa values by UV–visible spectroscopy and DFT calculations. *Journal of Chemical and Engineering Data*, 58(6), 1706–1716. <https://doi.org/10.1021/je400153r>
- de Lima, F.S., Kurozawa, L.E. and Ida, E.I. (2014). The effects of soybean soaking on grain properties and isoflavones loss. *LWT - Food Science and Technology*, 59(2), 1274-1282. <https://doi.org/10.1016/j.lwt.2014.04.032>
- Failisnur, Firdausni and Silfia (2015). The effect of processing on physical and chemical properties of soybean powder. *Jurnal Litbang Industri*, 5(1), 37-43. <https://doi.org/10.24960/jli.v5i1.664>
- Fathinatullabibah, Kawiji and Khasanah, L.U. (2014). Stability of anthocyanins from teak leaf extract (*Tectona grandis*) against pH and temperature treatments. *Jurnal Ilmu dan Inovasi Pangan*, 3(1), 5163. <https://doi.org/10.29313/jiff.v3i1.5163>
- Ghoreishi, S.M. and Shahrestani, R.G. (2009). Subcritical water extraction of mannitol from olive leaves. *Journal of Food Engineering*, 93(4), 474-481. <https://doi.org/10.1016/j.jfoodeng.2009.02.015>
- Jiménez-Monreal, A.M., García-Diz, L., Martínez-Tomé, M., Mariscal, M. and Murcia, M.A. (2009). Influence of cooking methods on antioxidant activity of vegetables. *Journal of Food Science*, 74(3), H99-H103. <https://doi.org/10.1111/j.1750-3841.2009.01091.x>
- Ju, Z.Y. and Howard, L.R. (2005). Subcritical water and sulfured water extraction of anthocyanins and other phenolics from dried red grape skin. *Journal of Agricultural and Food Chemistry*, 53(9), 3620–3624. <https://doi.org/10.1021/jf047974q>
- King, J.W. and Grabiell, R.D. (2007). Isolation of polyphenolic compounds from fruits or vegetables utilizing sub-critical water extraction (United States Department of Agriculture patents No. 7208181). USA: United States Patent and Trademark Office (UPTO)
- King, J.W., Gabriel, R.D. and Wightman, J.D. (2009). Subcritical Water Extraction of Anthocyanins from Fruit Berry Substrates. Supercritical Fluid Facility. Los Alamos National Laboratory C.ACT Group Chemistry Division. Los Alamos. USA.
- Kinyanjui, P.K., Njoroge, D.M., Makokha, A.O., Christiaens, S., Ndaka, D.S. and Hendrickx, M. (2015). Hydration properties and texture fingerprints of easy- and hard-to-cook bean varieties. *Food Science and Nutrition*, 3(1), 39-47. <https://doi.org/10.1002/fsn3.188>
- Kusumawati, I., Astawan, M. and Prangdimurti, E. (2020). Production process and characteristics of tempeh from broken soybeans. Bogor, Indonesia: Food Technology Study Program, Postgraduate School, Bogor Agricultural Institute.
- Liu, W.-T., Huang, C.-L., Liu, R., Yang, T.-C., Lee, C.-L., Tsao, R. and Yang, W.-J. (2023). Changes in isoflavone profile, antioxidant activity, and phenolic contents in Taiwanese and Canadian soybeans during tempeh processing. *LWT-Food Science and Technology*, 186, 115207. <https://doi.org/10.1016/j.lwt.2023.115207>
- Navajas-Porras, B., Pérez-Burillo, S., Valverde-Moya, Á.J., Hinojosa-Nogueira, D., Pastoriza, S. and Rufián-Henares, J.Á. (2020). Effect of cooking methods on the antioxidant capacity of plant foods submitted to in vitro digestion–fermentation. *Antioxidants*, 9(12), 1312. <https://doi.org/10.3390/antiox9121312>
- Ng, Z.X., Chai, J.W. and Kuppusamy, U.R. (2011). Customized cooking method improves total antioxidant activity in selected vegetables. *International Journal of Food Sciences and Nutrition*, 62(2), 158–163. <https://doi.org/10.3109/09637486.2010.526931>
- Richter, P., Toral, M.I. and Toledo, C. (2006). Subcritical water extraction and determination of nifedipine in pharmaceutical formulations. *Journal of AOAC International*, 89(2), 365-368. <https://doi.org/10.1093/jaoac/89.2.365>
- Rompas, R.A., Edy, H.J. and Yudistira, A. (2012). Isolasi dan identifikasi flavonoid dari daun lamun (*Syringodium isoetifolium*). *Pharmakon*, 1(2), 59-63. <https://doi.org/10.35799/pha.1.2012.487> [In Bahasa Indonesia].
- Teixeira-Guedes, C.I., Oppolzer, D., Barros, A.I. and Pereira-Wilson, C. (2019). Impact of cooking method on phenolic composition and antioxidant potential of four varieties of *Phaseolus vulgaris* L. and *Glycine max* L. *LWT - Food Science and Technology*, 103, 238–246. <https://doi.org/10.1016/j.lwt.2019.01.004>
- Thummakomma, K., Prashanthi, M. and Rajeswari, K.

- (2018). Effect of cooking methods on bioactive compounds in vegetables. *International Journal of Chemical Studies*, 6(4), 3310–3315. <https://doi.org/10.13140/RG.2.2.35490.27844>
- Toda, T., Sakamoto, A., Takayanagi, T. and Yokotsuka, K. (2001). Changes in Isoflavone Compositions of Soybean during Soaking in Water. *Food Science and Technology Research*, 7(2), 171–175. <https://doi.org/10.3136/FSTR.7.171>
- Tsikrika, K., O'Brien, N. and Rai, D.K. (2019). The effect of high-pressure processing on polyphenol oxidase activity, phytochemicals, and proximate composition of Irish potato cultivars. *Foods*, 8(10), 517. <https://doi.org/10.3390/foods8100517>
- Urasopon, N., Hamada, Y., Asaoka, K., Pongmali, U. and Malaivijitnond, S. (2008). Isoflavone content of rodent diets and its estrogenic effect on vaginal cornification in *Pueraria mirifica*-treated rats. *ScienceAsia*, 34(4), 371–376. <https://doi.org/10.2306/scienceasia1513-1874.2008.34.371>
- Veda, S., Platel, K. and Srinivasan, K. (2010). Enhanced bioaccessibility of  $\beta$ -carotene from yellow-orange vegetables and green leafy vegetables by domestic heat processing. *International Journal of Food Science and Technology*, 45(10), 2201–2207. <https://doi.org/10.1111/j.1365-2621.2010.02385.x>
- Wang, N. and Daun, J.K. (2005). Determination of cooking times of pulses using an automated Mattson cooker apparatus. *Journal of the Science of Food and Agriculture*, 85(10), 1631–1635. <https://doi.org/10.1002/jsfa.2134>
- Wang, T.-Y., Li, Q. and Bi, K.-S. (2018). Bioactive flavonoids in medicinal plants: Structure, activity and biological fate. *Asian Journal of Pharmaceutical Sciences*, 13(1), 12–23. <https://doi.org/10.1016/j.ajps.2017.08.004>
- Yudiono, K. (2011). Anthocyanin extraction from purple sweet potato (*Ipomoea batatas* cv. Ayamurasaki) with subcritical water extraction technique. *Journal Food Technology: Scientific Information and Communication Media for Agricultural Technology*, 2(1),1-30.
- Yudiono, K., Ayu, W.C. and Susilowati, S. (2021). Antioxidant activity, total phenolic, and aflatoxin contamination in tempeh made from assorted soybeans (*Glycine max* L. Merrill). *Food Research*, 5 (3), 393-398. [https://doi.org/10.26656/fr.2017.5\(3\).655](https://doi.org/10.26656/fr.2017.5(3).655)
- Zhang, L., Qu, H., Xie, M., Shi, T., Shi, P. and Yu, M. (2023). Effects of different cooking methods on phenol content and antioxidant activity in sprouted peanut. *Molecules*, 28(12), 4684. <https://doi.org/10.3390/molecules28124684>