International Food Research Journal 29(4): 857 - 863 (August 2022)
Journal homepage: *http://www.ifrj.upm.edu.my*

Subcritical reactive extraction of shogaol and gingerol: Effect of time and temperature

[1]Yulianto, M. E., [2]Purwantisari, S., [3]Hartati, I., [1]Nisa, Q. A. K. and [1]*Nyamiati, R. D.

[1]*Department of Chemical Engineering Technology, Faculty of Industrial Technology, Pembangunan Nasional "Veteran" Yogyakarta University, Yogyakarta 55283, Indonesia*
[2]*Department of Biology, Faculty of Mathematics and Natural Sciences, Diponegoro University, 50275 Indonesia*
[3]*Chemical Engineering Department, Faculty of Engineering, Wahid Hasyim University, 50236 Indonesia*

Article history

Received:
19 August 2021
Received in revised form:
14 October 2021
Accepted:
14 December 2021

Keywords

shogaol,
gingerol,
Zingiber officinale,
subcritical water,
reactive extraction

Abstract

Gingerol and shogaol are two bioactive compounds of ginger which exhibit several positive effects on human health. The conventional method for shogaol preparation is considered ineffective because it causes losses to the environmental system, and the efficiency of the process is low. Reactive extraction with subcritical water is a method that is considered environmentally friendly for the separation of slightly polar components without using organic solvents. The advantage of the reactive extraction process with subcritical water is that it does not require a catalyst because the process is able to form hydronium ions which can act as a catalyst to convert shogaol into gingerol. The effect of time and temperature on the yields of subcritical water reactive extraction of gingerol and shogaol were investigated in the present work. Experiments were carried out at a fixed pressure of 2 bar, and a varied temperature of reactive extraction from 130 to 140°C. Ginger and shogaol contents were analysed from the samples every 10 min. The chemical profiling of the resulting ginger extracts was performed using HPLC-MS. Results showed that the best subcritical water process was at 2 bar, 130°C, and 20 min; shogaol concentration increased to 15.345%, and gingerol to 5.113%. For the reactive extraction time of longer than 20 min, the shogaol concentration of the extract decreased. When temperature was above 120°C, and water hydrogen bonds weakened, thus resulting in high amounts of ionisation products of subcritical water.

DOI
https://doi.org/10.47836/ifrj.29.4.12

Introduction

In 2021, Indonesia imported 95% of the raw material needed for national drug production at a value of 11.4 trillion. The increasing independence in health sciences was seen with the improvement of the production of medicinal raw materials from the biodiversity of Indonesia, and also the production of the drugs themselves (Thomson *et al*., 2002; Anisa *et al*., 2014).

Ginger is of the family Zingiberaceae and genus *Zingiber*, and has been consumed as herbs and spices for a long time (Han *et al*., 2013). Ginger has shown biological activities such as anti-inflammatory, antioxidative, anticancer, and antimicrobial (Mao *et al*., 2019). The main bioactive components of ginger are gingerol, shogaol (Bak *et al*., 2012), paradol, and zingerone which have various health effects such as antiosteoporosis, antioxidant, and anticancer (Eikani *et al*., 2007; Ali *et al*., 2008; Dhanik *et al*., 2017). 6-gingerol and 6-shogaol are bioactive compounds that have a similar chemical structure and useful bioactive properties, with 6-shogaol showing stronger power (Braga, 2019). 6-shogaol has greater effectiveness than 6-gingerol such as antioxidant (Bhattarai *et al*., 2001; Eikani *et al*., 2007; Pawar *et al*., 2011; Dhanik *et al*., 2017; Aryanti *et al*., 2018), anti-inflammatory (Eikani *et al*., 2007; Pan *et al*., 2008; Sang *et al*., 2009), anti-platelet aggregation (Shih *et al*., 2014), reducing pain due to muscle contraction in the body (Han *et al*., 2013), inhibiting the formation of colorectal cancer precursors (Pan *et al*., 2008; Jeevani Osadee Wijekoon *et al*., 2011), reducing the formation of cancer cells in the ovaries (Rhode *et al*., 2007; Svarc-Gajic *et al*., 2017), and reducing the source of breast and lung cancer cells (Wu *et al*., 2015). The bioactive compounds from ginger extract can be commercially

*Corresponding author.
Email: retno.dwinyamiati@upnyk.ac.id

produced and used for medicines and food supplements (Teng *et al*., 2019). Cheng *et al*. (2011) conducted a study on ginger extraction to obtain 6-shogaol without using a catalyst, and the conversion was still low of only 40% at 120°C for 4 h (Cheng *et al*., 2011). Guo *et al.* (2017) studied the conversion of gingerol to shogaol with the help of microwaves combined with acidic food seasonings, and the conversion rate was also relatively low at 58.6% at 140°C, and a microwave power of 1,000 W.

A good solvent for extraction is non-toxic, easy to obtain, abundant in nature, can be recycled, has purity close to alcohol (Syahdi *et al*., 2020), and has low viscosity and surface tension. Conventional solvent extraction processes are still widely used on a large scale in the industry (Chan *et al*., 2009; Dyachok, 2010). Ginger has been extracted using conventional extraction techniques such as ultrasound with microwaves, and Soxhlet using solvents such as acetone, methanol, and ethanol (Svarc-Gajic *et al*., 2017; Handayani *et al*., 2020). However, conventional extraction has disadvantage, such as it requires the addition of a catalyst, which increases the operational costs and purification burden (Guo *et al*., 2017).

Hydrothermal extraction process could be a better alternative such as the reactive extraction process using water solvent under subcritical conditions. Thermodynamically, water that is kept in a liquid condition below its critical point, in the temperature range of 100 - 374°C, with a pressure of less than 22 MPa, is referred to as subcritical water (Teo *et al*., 2010). Reactive extraction process using subcritical water as a green solvent has attracted the attention of many researchers. The use of subcritical water above its normal boiling point of 100°C as a solvent is often referred to as hot compressed water (HCW) (Sunphorka *et al*., 2012). Extraction with HCW has been carried out by many researchers in the extraction of compounds from cumin (Eikani *et al*., 2007), *Centella asiatica* (Svarc-Gajic *et al*., 2017), *Thymbra spicata*, melon biter, oregano, and *Curcuma domestica* Val (Yulianto *et al*., 2018a; 2018b).

When water temperature is increased, there is an increase in the dielectric constant with sufficient pressure to maintain the water in liquid phase. At high temperatures, there is a decrease in the dielectric constant from 80 to 27 at 25°C, pressure of 5 bar, and the dielectric constant is equivalent to the dielectric constant of methanol (ε = 33) and ethanol (ε = 24) at 25°C. As such, water behaves like an organic solvent that can dissolve various analytes, and has a low to medium polarity (Anisa *et al*., 2014). In subcritical area (100 - 374°C), the ionisation constant (kW) also increase with increasing temperature. At temperatures above 120°C, the water hydrogen bonds weaken, thus resulting in high amounts of ionisation products of subcritical water. Another role of subcritical water is that it can perform as a catalyst (Paramita *et al*., 2019). Weak hydrogen bonds result in auto-ionisation of water into acid catalyst hydronium ion (H_3O^+) and base catalyst hydroxide ion (OH^-). It is also stated that subcritical water can reduce the activation energy (Lachance *et al*., 2008).

The development of a reactive extraction process with subcritical water is believed to be able to convert 6-gingerol to 6-shogaol maximally with a fairly high level of shogaol selectivity. The use of subcritical water as solvent in the reactive extraction process, which is considered to reduce viscosity and surface tension, can encourage mass transfer rates, absorption into matrix particles, and increase selectivity, which also increase the diffusivity up to 10 times (Yulianto *et al*., 2018a; 2018b). The experiment was carried out under a pressure of 35 bar. The optimal temperatures determined for the highest yields of 6-gingerol and 6-shogaol were 130 and 170°C, respectively. The present work aimed to determine the effect of temperature and reactive extraction time on the yield of ginger shogaol and gingerol using subcritical water. Analysis of the samples was done using HPLC-MS (high-performance liquid chromatography-mass spectrometry).

Materials and methods

Materials

Emprit ginger (length: 5 - 8 cm; age: 8 - 9 months) was obtained from UMKM Tiga Dara Ungaran. Methanol, potassium hydroxide, phenolphthalein indicator, and 6-shogaol and 6-gingerol standards were purchased from Sigma Aldrich. Distilled water was used as a subcritical water reactive extraction solvent. Nitrogen was used to remove air and dissolved oxygen.

Methods

Variable preparation

Variables that were varied were temperature and reactive extraction time (Paramita *et al*., 2019). Reactive extraction temperatures were set at 130 and

140°C. The reactive extraction times were set at 10, 20, and 40 min, with phase contact between the solute and the continuous phase quite wide. Experiments were carried out at a fixed pressure of 2 bar. Samples were taken every 10 min, and analysed for their gingerol and shogaol contents.

Subcritical water reactive extraction

Ginger powder (70 g) was added with 700 mL of distilled water. The mixture was then placed in a stainless tube cell with lid. Liquefied nitrogen gas was then inserted into the cell for 2 min to purge the oxygen. The valve of the reaction chamber was used to control the pressure chamber, and the temperature was set in accordance with the experimental design. The heating process took 3 - 5 min to reach the desired temperature. The initial time of the reactive extraction process was taken when the temperature reached under certain conditions in accordance with the experimental design. The extract was cooled in a cooling cell at 25°C and 1 MPa for 1 min.

HPLC-MS analysis

The chemical profile of shogaol and gingerol from ginger extracts was analysed using HPLC-MS (Svarc-Gajic *et al*., 2017). A UPLC-QToF-MS/MS (water) system with Acquity UPLC BEH C18 1.7 µm, 2.1 × 50 mm column was used. The analysis was performed by applying two types of effluents. The first one was water with 0.1% of formic acid, and the second one was acetonitrile with 0.1% of formic acid. The injection volume was 5 µL at 40°C and 0.3 mL/min flowrate. The MS of the LC used was XEVO - G2QTOF (water) with positive ESI in resolution mode. Masslynk software version 4.1 was used to analyse all the data collected.

Statistical analysis

Statistical analysis was conducted using response surface methodology (RSM) as detailed by Paramita *et al.* (2019). Of the 115 to 135°C temperature range, the best results were obtained at 125°C. Therefore, this study was optimised by narrowing the temperature in the range of 130 and 140°C.

Results and discussion

The effect of subcritical water reactive extraction time is shown in Figure 1. It can be seen that along the reactive extraction time of up to 20 min, the shogaol concentration was increasing. This phenomenon was conceivable since the longer the phase contact time between the dispersed phase and the continuous phase (subcritical water), the more the dispersed phase dragged into the continuous phase. Therefore, in the process of obtaining shogaol, there was an increase due to the higher solubility characteristics of subcritical water and a dielectric constant decrease of water with increasing temperature. Furthermore, Figure 1 also shows that at the reactive extraction time of longer than 20 min, the shogaol concentration decreased. The decrease in shogaol was due to its degradation into paradol (Thomson *et al*., 2002; Rahmani *et al*., 2014).

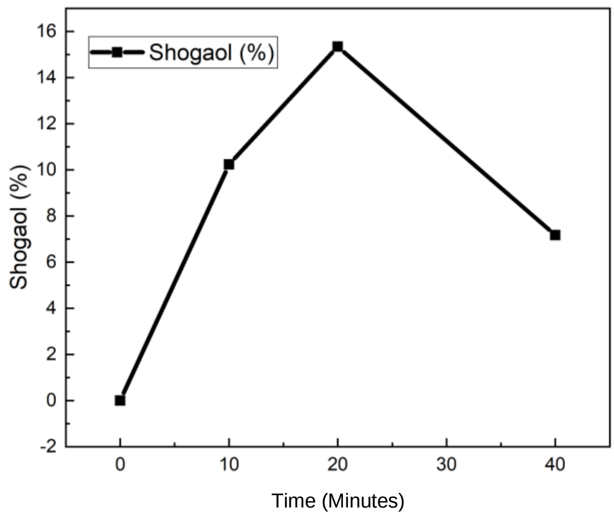

Figure 1. Effect of shogaol production under different reactive extraction times at 130°C.

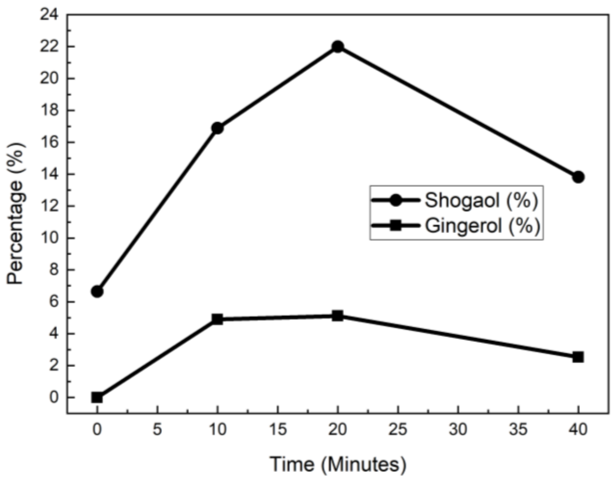

Figure 2. Effect of shogaol and gingerol production under different reactive extraction times.

Figure 2 shows the curve of gingerol yield versus shogaol against reactive extraction time using subcritical water. The analysis of both gingerol and shogaol contents was conducted with the subcritical water reactive extraction performed at 130°C, and reactive extraction duration up to 40 min with the interval time of 10 min. The longer the reactive extraction time, the more gingerol and shogaol were obtained. However, after 20 min, the gingerol and shogaol yields decreased. This is because gingerol underwent dehydration to become shogaol, while some of the shogaol was degraded into paradol.

The conversion of shogaol due to extended contact to heat is mentioned in a study conducted by Andersson *et al*. (2003). They reported that shogaol, an aromatic group found in ginger, is degraded by long heat exposure at reactive extraction temperatures above 170 - 190°C. This causes reactive extraction at temperatures above 150°C to be ineffective for ginger extraction (Andersson *et al*., 2003). It is also mentioned that by controlling both temperature and duration of the separation process, the ginger extraction process to obtain gingerol and shogaol can be carried out selectively (Ko *et al*., 2019), and it can be seen in Figure 3.

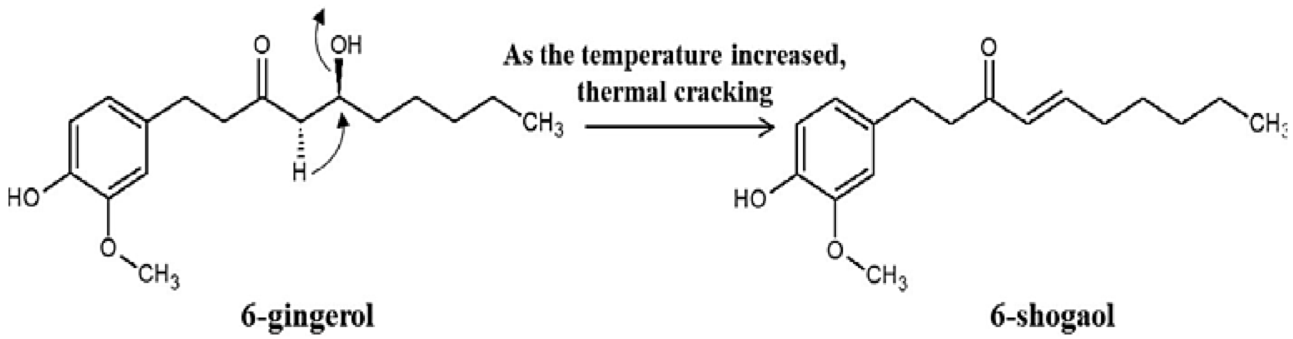

Figure 3. The conversion of 6-gingerol to 6-shogaol by temperature-dependent thermal cracking.

In Figure 4, the effect of temperature on shogaol production at 10 min of subcritical water reactive extraction is presented. It can be seen that the higher the temperature, the higher the percentage of shogaol. This agrees with Ko *et al.* (2019) who reported that along with the increase in time and temperature (110 - 130°C), the content of 6-gingerol decreased while the content of 6-shogaol increased. However, the yield of the product decreased with increasing reactive extraction temperature at 150 - 190°C (Ko *et al*., 2019).

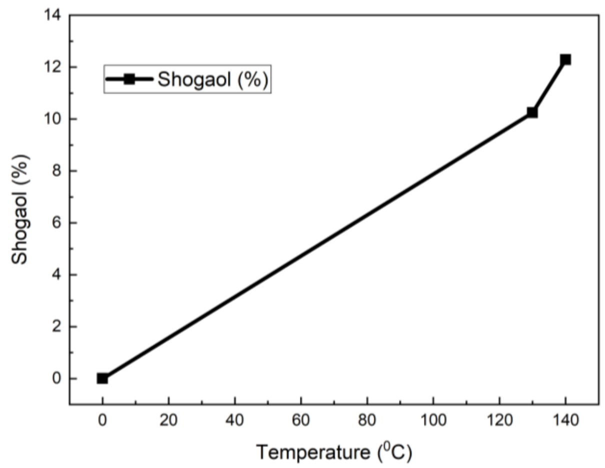

Figure 4. Effect of temperature on shogaol production in 10 min.

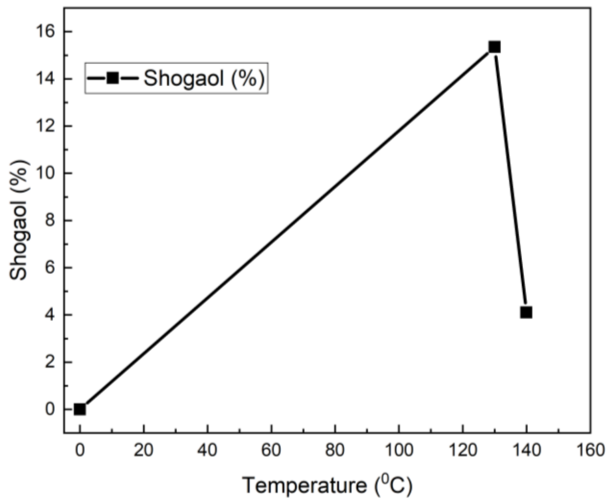

Figure 5. Effect of temperature on shogaol production in 20 min.

However, at over 20 min in Figure 5, shogaol decreased further as the reactive extraction temperature increased at temperatures above 130°C. It can be seen that the recovery of shogaol has increased. This is because at temperatures above 130°C, subcritical water changes its function into a non-polar solvent, as well as a catalyst due to the higher number of ionisation product of subcritical water than that of water. The results of the chromatogram on the extraction variable of 130°C and 20 min can be seen in Figure 6. This also agrees with Ko *et al.* (2019) in that double bonds in water compounds can make compounds more stable at subcritical temperatures (Cheng *et al*., 2011). The increase in gingerol and shogaol yields occurred at temperatures above 130°C. Subcritical water as solvent experiences non-polar properties due to a decrease in the dielectric constant. It is reported that at reactive extraction temperature above 150°C, the water hydrogen bonds become weakened and followed by the production of hydronium and hydroxide ions from the auto-ionisation process of water (Lachance *et al*., 2008).

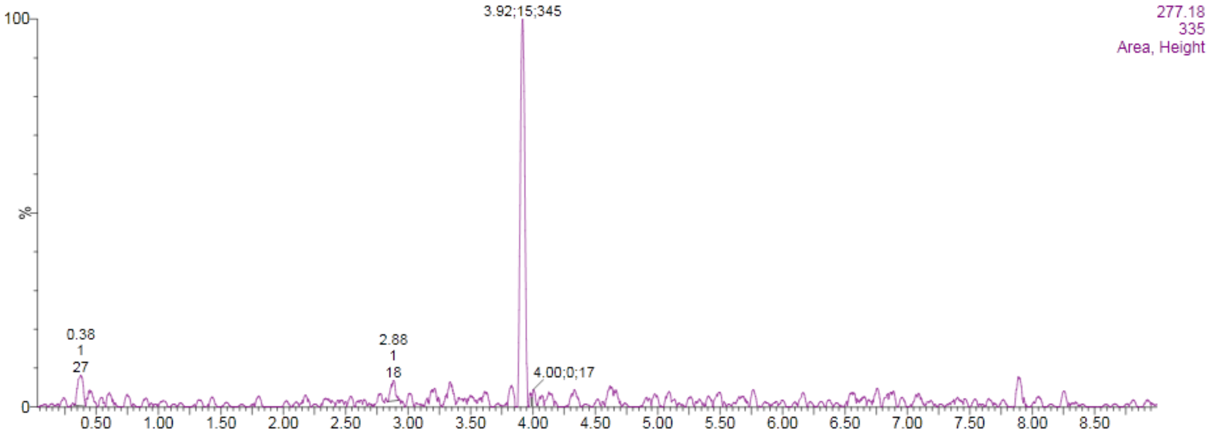

Figure 6. Chromatogram of shogaol and gingerol at 130°C, 2 bar, and 20 min.

Increasing the reactive extraction temperature causes the value of water dielectric (ε) of 80 at 25°C decreases to 27 at 25°C, meanwhile the range of methanol and ethanol was ε = 33 and 24, respectively (Eikani *et al*., 2006). Weak hydrogen bonds result in auto-ionisation of water into acid catalyst (hydronium ion, H_3O^+) and base catalyst (hydroxide ion, OH^-). It is also stated that subcritical water can reduce the activation energy (Lachance *et al*., 2008). The dissociation constant (kW) of water increases from 1.0 × 10 - 14, at 25°C, to a maximum value of 4.9 × 10 - 12 at 250°C. This indicates that the pH changes from about 7.0 to 5.5 (Eikani *et al*., 2006).

Conclusion

Gingerol and shogaol are two of ginger's bioactive components with a myriad of positive effects on human health. The application of subcritical water method is possible for the simultaneous conversion of shogaol from gingerol as well as the isolation of both compounds. The present work determined the effect of temperature and reactive extraction time on the yields of shogaol and gingerol using subcritical water. The best reactive extraction time was found to be up to 20 min, where the shogaol concentration of the extracts increased to 15.345%. When the reactive extraction time was prolonged to more than 20 min, the shogaol concentration of the extract decreased due to its degradation into paradol. The best temperature found was 130°C since at temperature above 120°C, the water hydrogen bonds weaken, thus resulting in high amounts of ionisation products of subcritical water.

Acknowledgement

The present work was financially supported by the Higher Education Leading Applied Research Program (PTUPT) 2020 and DRPM - Ministry of Research and Technology / National Research and Innovation Agency.

References

Ali, B. H., Blunden, G., Tanira, M. O. and Nemmar, A. 2008. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): A review of recent research. Food and Chemical Toxicology 46: 409-420.

Andersson, T., Hartonen, K., Hyötyläinen, T. and Riekkola, M. L. 2003. Stability of polycyclic aromatic hydrocarbons in pressurised hot water. Analyst 2: 150-155.

Anisa, N. I., Azian, N., Sharizan, M. and Iwai, Y. 2014. Temperature effects on diffusion coefficient for 6-gingerol and 6-shogaol in subcritical water extraction. Journal of Physics - Conference Series 1: 160-165.

Aryanti, N., Nafiunisa, A., Bella, N., Sanjaya, R., Wardhani, D. H. and Kumoro, A. C. 2018.

Kinetics of ultrasound-assisted extraction of anthocyanin from purple roselle calyces under different PH conditions. Chemistry and Chemical Technology 4: 523-528.

Bak, M. J., Ok, S., Jun, M. and Jeong, W. S. 2012. 6-shogaol-rich extract from ginger up-regulates the antioxidant defense systems in cells and mice. Molecules 17(7): 8037-8055.

Bhattarai, S., Van Tran, H. and Duke, C. C. 2001. The stability of gingerol and shogaol in aqueous solutions. Journal of Pharmaceutical Sciences 90(10): 1658-1664.

Braga, S. S. 2019. Ginger: Panacea or consumer's hype? Applied Sciences 8: 1-16.

Chan, S. W., Lee, C. Y., Yap, C. F., Wan Aida, W. M. and Ho, C. W. 2009. Optimisation of extraction conditions for phenolic compounds from limau purut (*Citrus hystrix*) peels. International Food Research Journal 2: 203-213.

Cheng, X. L., Liu, Q., Peng, Y. B., Qi, L. W. and Li, P. 2011. Steamed ginger (*Zingiber officinale*): Changed chemical profile and increased anticancer potential. Food Chemistry 4: 1785-1792.

Dhanik, J., Arya, N., Nand, V. and Jyotsna Dhanik, C. 2017. A review on *Zingiber officinale*. Journal of Pharmacognosy and Phytochemistry 3: 174-184.

Dyachok, V. 2010. Extraction process of intracellular substance. Chemistry and Chemical Technology 12: 163-166.

Eikani, M. H., Golmohammad, F., Mirza, M. and Rowshanzamir, S. 2007. Extraction of volatile oil from cumin (*Cuminum cyminum* L.) with superheated water. Journal of Food Process Engineering 30: 255-266.

Eikani, M. H., Golmohammad, F., Rowshanzamir, S. and Namdar, H. K. 2006. Subcritical water extraction of essential oils from coriander seeds (*Coriandrum sativum* Mill.) Journal of Food Engineering 80: 735-740.

Guo, J. B., Fan, Y., Zhang, W. J., Wu, H., Du, L. M. and Chang, Y. X. 2017. Extraction of gingerols and shogaols from ginger (*Zingiber officinale* Roscoe) through microwave technique using ionic liquids. Journal of Food Composition and Analysis 62: 35-42.

Han, Y. A., Song, C. W., Koh, W. S., Yon, G. H., Kim, Y. S., Ryu, S. Y., … and Lee, K. H. 2013. Anti-inflammatory effects of the *Zingiber officinale* Roscoe constituent 12-dehydrogingerdione in lipopolysaccharide-stimulated Raw 264.7 cells. Phytotherapy Research 27(8): 1200-1205.

Handayani, R., Bangun, A., Deborah, P. D. and Mun'im, A. 2020. Optimization of microwave- and ultrasonic-assisted extraction of mahkota dewa (*Phaleria macrocarpa* [Scheff.] Boerl.) fruit pulp. International Journal of Applied Pharmaceutics 12: 32-37.

Jeevani Osadee Wijekoon, M. M., Karimand, A. A., and Bhat, R. 2011. Evaluation of nutritional quality of torch ginger (*Etlingera elatior* Jack.) inflorescence. International Food Research Journal 18: 1415-1420.

Ko, M. J., Nam, H. H. and Chung, M. S. 2019. Conversion of 6-gingerol to 6-shogaol in ginger (*Zingiber officinale*) pulp and peel during subcritical water extraction. Food Chemistry 270: 149-155.

Lachance, R. P., Fro, M., Antal, M. J., Peterson, A. A. and Tester, J. W. 2008. Thermochemical biofuel production in hydrothermal media : A review of sub- and supercritical water technologies. Energy and Environmental Science 5: 32-65.

Mao, Q. Q., Xu, X. Y., Cao, S. Y., Gan, R. Y., Corke, H., Beta, T. and Li, H. B. 2019. Bioactive compounds and bioactivities of ginger (*Zingiber officinale* Roscoe). Foods 8: 1-21.

Pan, M. H., Hsieh, M. C., Kuo, J. M., Lai, C. S., Wu, H., Sang, S. and Ho, C. T. 2008. 6-Shogaol induces apoptosis in human colorectal carcinoma cells *via* ROS production, caspase activation, and GADD 153 expression. Molecular Nutrition and Food Research 52: 527-537.

Paramita, V., Yulianto, M. E., Hartati, I., Bahrudin, U. and Wisnubroto, R. 2019. Optimization on subcritical fluid extraction of zingiberene. Journal of Vocational Studies on Applied Research 1: 18-21.

Pawar, N., Pai, S., Nimbalkar, M. and Dixit, G. 2011. RP-HPLC analysis of phenolic antioxidant compound 6-gingerol from different ginger cultivars. Food Chemistry 126: 1330-1336.

Rahmani, A. H., Al Shabrmi, F. M. and Aly, S. M. 2014. Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities. International Journal of Physiology, Pathophysiology and Pharmacology 6: 125-136.

Rhode, J., Fogoros, S., Zick, S., Wahl, H., Griffith, K. A., Huang, J. and Rebecca, J. R. 2007. Ginger inhibits cell growth and modulates angiogenic factors in ovarian cancer cells. BMC Complementary and Alternative Medicine 7: article no. 44.

Sang, S., Hong, J., Wu, H., Liu, J., Yang, C. S., Pan, M. H., … and Ho, C. T. 2009. Increased growth inhibitory effects on human cancer cells and anti-inflammatory potency of shogaols from *Zingiber officinale* relative to gingerols. Journal of Agricultural and Food Chemistry 57: 10645-10650.

Shih, H. C., Chern, C. Y., Kuo, P. C., Wu, Y. C., Chan, Y. Y., Liao, Y. R., … and Wu, T. S. 2014. Synthesis of analogues of gingerol and shogaol, the active pungent principles from the rhizomes of *Zingiber officinale* and evaluation of their anti-platelet aggregation effects. International Journal of Molecular Sciences 15: 3926-3951.

Sunphorka, S., Chavasiri, W., Oshima, Y. and Ngamprasertsith, S. 2012. Protein and sugar extraction from rice bran and de-oiled rice bran using subcritical water in a semi-continuous reactor: Optimization by response surface methodology. International Journal of Food Engineering 8: 105-110.

Svarc-Gajic, J., Cvetanovic, A., Segura-Carretero, A., Linares, I. B. and Mašković, P. 2017. Characterisation of ginger extracts obtained by subcritical water. Journal of Supercritical Fluids 123: 92-100.

Syahdi, R. R., Nadyana, R., Putri, R. H., Santi, R. and Mun'im, A. 2020. Application of green extraction methods to resveratrol extraction from peanut (*Arachis Hypogaea* L.) skin. International Journal of Applied Pharmaceutics 12: 38-42.

Teng, H., Seuseu, K. T., Lee, W. Y. and Chen, L. 2019. Comparing the effects of microwave radiation on 6-gingerol and 6-shogaol from ginger rhizomes (*Zingiber officinale* Rosc). PLoS One 14: 1-16.

Teo, C. C., Tan, S. N., Yong, J. W. H., Hew, C. S. and Ong, E. S. 2010. Pressurized hot water extraction (PHWE). Journal of Chromatography 1217: 248-2494.

Thomson, M., Al-Qattan, K. K., Al-Sawan, S. M., Alnaqeeb, M. A., Khan, I. and Ali, M. 2002. The use of ginger (*Zingiber officinale* Rosc.) as a potential anti-inflammatory and antithrombotic agent. Prostaglandins Leukotrienes and Essential Fatty Acids 67: 475-478.

Wu, C. H., Hong, B. H., Ho, C. T. and Yen, G. C. 2015. Targeting cancer stem cells in breast cancer: Potential anticancer properties of 6-shogaol and pterostilbene. Journal of Agricultural and Food Chemistry 63: 2432-2441.

Yulianto, M. E., Paramita, V., Hartati, I. and Amalia, R. 2018a. Response surface methodology of pressurized liquid water extraction of curcumin from *Curcuma domestica* val. Rasayan. Journal of Chemistry 11: 1564-1571.

Yulianto, M. E., Paramita, V., Hartati, I. and Handayani, D. 2018b. Hot compressed water extraction of curcumin from *Curcuma domestica* Val. AIP Conference Proceedings 1977: 1130-1143.