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

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Abstract
Ginger 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.

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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

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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 (H₃O⁺) 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.](image1)

![Figure 2. Effect of shogaol and gingerol production under different reactive extraction times.](image2)
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](image3.png)

**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](image4.png)

**Figure 4.** Effect of temperature on shogaol production in 10 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).

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, H₃O⁺) 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.

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