

Oleoresin Extraction from *Zingiber officinale* Using Hot Compressed Water Extraction

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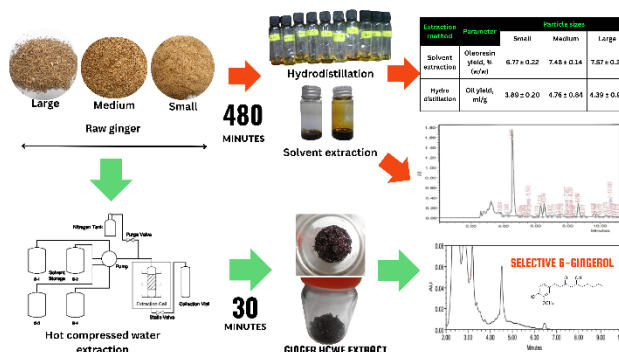
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Abstract: Ginger is traditionally processed into two main products: ginger oleoresin and oil using solvent extraction and hydro-distillation, respectively. However, these methods have limitations, including the use of organic solvents, long extraction times, and poor selectivity. In this study, hot compressed water extraction (HCWE) was evaluated as an alternative, and its performance was compared with conventional methods in terms of yield, physical characteristics, and concentration of key bioactive compounds. The HCWE was conducted at a constant pressure of 3.5 MPa and varying temperatures between 130°C and 200°C. The resulting extracts were analyzed using High-Performance Liquid Chromatography (HPLC) to quantify 6-gingerol, 6-shogaol, and 10-gingerol concentration. HCWE process at temperature of 140°C, pressure of 3.5 MPa and 30 minutes extraction time obtains higher extraction yield of 10.37 ± 0.33 % (g/g) in comparison with solvent extraction for 480 minutes at 7.57 ± 0.31 % (g/g). But it resulted in lower concentrations of 6-gingerol at 1957.22 ± 2.55 µg/g and undetectable levels of 6-shogaol. As temperature increased to 170°C, the concentration of 6-gingerol decrease to 851.95 ± 2.34 µg/g and 6-shogaol concentration increase to 1135.23 ± 1.18 µg/g demonstrating HCWE's temperature-driven selectivity. Above 170 °C, both compounds degraded significantly, and the extract quality declined. The HCWE extract resembled ginger oleoresin in texture and aroma, suggesting its suitability for similar applications. In contrast, solvent extraction produced a broader range of compounds but lacked selectivity. In conclusion, this study demonstrates that the HCWE process is a rapid, organic solvent-free extraction method which not only reduces processing time by more than 80 % compared with conventional solvent extraction, but also provides temperature-driven selectivity, underscoring its potential for greener, industrial-scale applications in functional food and phytochemical production. These findings highlight the promise of HCWE as a sustainable approach for phytochemical extraction and functional food development.



Keywords: selective extraction, green process, oleoresin like extract, 6-gingerol, 6-shogaol

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1 Introduction

GINGER (*Zingiber officinale*) is widely recognized for its medicinal properties, including anticancer, antioxidant, and anti-inflammatory effects ^{1,2}. These therapeutic benefits are primarily attributed to bioactive compounds from the gingerol, shogaol, and paradol families, with 6-gingerol, 10-gingerol, and 6-shogaol identified as the most potent pharmacologically active constituents. Among them, 6-gingerol, abundant in fresh ginger has demonstrated cardioprotective, antioxidant, antiproliferative, and apoptotic activities ^{3,4}. Meanwhile, 10-gingerol exhibits notable antioxidant and attenuated Lipopolysaccharide-induced inflammation ⁵, and 6-shogaol, predominant in dried ginger, has shown neuroprotective and anticancer effects ⁶. Pharmacokinetic data by Zhang et al., 2022⁷ showed that about one-third of 6-gingerol was converted into 6-gingerdiols, while over 90 % of 6-shogaol was metabolized into reduction and cysteine-conjugated metabolites which can benefit the asthma patient.

Ginger is commonly processed into two main products namely ginger oleoresin and ginger oil which differ significantly in their physical appearance, chemical composition, and extraction methods. Ginger oleoresin, conventionally obtained via solvent extraction, contains non-volatile phenolic compounds like gingerols and shogaols. In contrast, ginger oil, conventionally extracted by hydro-distillation, is rich in volatile constituents such as zingiberene, β -sesquiphellandrene, and α -curcumene ⁸ and typically constitute 1 to 3 % of ginger's total weight. Both process, solvent extraction and hydrodistillation required long extraction and the using of the organic solvent. Therefore, various alternatives have been introduced to improve the quality of ginger products, particularly regarding the concentration of bioactive compounds such as gingerol, by developing more efficient extraction processes ⁹. These include advanced methods such as microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), ultrasound-assisted extraction (UAE), pulsed electric field (PEF) extraction, and enzyme-assisted extraction (EAE), which offer higher yields and are more eco-friendly ⁹. One of the eco-friendly methods is hot compressed water extraction (HCWE), which essentially applies the principles of the PLE process but uses water as the solvent. Unlike traditional extraction methods, HCWE employs water under subcritical conditions at temperatures ranging from 100°C to 374°C under elevated pressure which enhances its solvating power due to changes in dielectric constant, viscosity, and surface tension. This method has been increasingly explored for extracting interest compound and bioactive from various matrix including palm oil¹⁰, brewer spent grains¹¹, *Phyllanthus tenellus* Roxb.¹², cinnamon bark¹³, agarwood¹⁴, caraway¹⁵, coriander seed¹⁶, noni fruits¹⁷ and propolis¹⁸. It is also being utilized for ginger related products, but most researchers are focus on kinetic and solubility study¹⁹, degradation study²⁰, phytochemical and cytotoxic activity study²¹ etc. And there is limited study focus on the physical characteristic of the extract including odor and color and its relation to the ginger bioactive compound. In HCWE process, temperature is considered as most influential parameter in extraction efficiency²². Meanwhile, the operating pressure has a minimal direct effect on the extraction efficiency, but it is essential to maintain water in its liquid state under subcritical condition and excessively high pressures increase equipment complexity and operational costs²². Therefore, in this study, the ability of HCWE process is explore for the ginger extraction process either for the ginger oil or oleoresin. The physical characteristic of ginger extract extracted using HCWE is also discussed and the effect of the temperature on the ginger yield and bioactive concentration is explained. The study also assesses the influence of temperature on yield and quality of the product. The performance of HCWE is benchmarked against conventional methods; ethanol-based solvent extraction and hydro-distillation, to evaluate its efficiency in extracting both volatile and non-volatile ginger bioactive.

2 Experimental Procedures

2.1 Materials

Fresh matured ginger rhizome was collected from Kota Kinabalu, Sabah. The rhizome was dried at 100-150°C for 8 hours before being grinded and vacuum packed in sealed bags prior to experimental process. The ginger standards of 6-gingerol, 10-gingerol and 6-shogaol were purchased from ChromaDex Inc, CA, USA. Acetonitrile and methanol were of HPLC grade, supplied by MERCK, Germany. Distilled water was used in the hot compressed water extraction (HCWE) and hydro distillation.

2.2 Pre-treatment, solvent extraction and hydro distillation

Sample pre-treatment process for ginger raw material involving the sieving process was conducted for sample standardization. The samples are sieved into three different sizes using the sieve ASTM E-11 (Fisher Scientific, USA) as shown in **Table 1**.

The screening was conducted using solvent extraction and hydro-distillation to investigate the effect of mean particle size. Hydro distillation is conducted with water to sample ratio 7.5:1(mL/g) and condenser temperature of 10°C for 8

hours extraction. The oil extracted was collected using the dean stark hydro distillation apparatus and measure for oil volume, v_{oil} . The oil yield (mL/g) was calculated using eq. 1.

$$\text{Oil yield (\%)} \frac{\text{mg}}{\text{g}} \times 100 = \frac{V_{oil}}{W_s} \times 100 \quad (1)$$

where, v_{oil} is ginger oil volume in millilitre (mL) and W_s is raw ginger weight in gram (g).

Solvent extraction was performed using ethanol as the solvent, with a solvent-to-sample ratio of 4:1 (mL/g) and a condenser temperature of 10 °C, for an extraction duration of 8 hours. Solvent removal was carried out in a 250 mL round-bottom flask using a rotary evaporator (Buchi R-205, Switzerland) set at a vacuum pressure of 175 mbar, a water bath temperature of 40 °C, a condenser temperature of 10 °C, and a rotation speed of 120 rpm. The percentage yield of oleoresin was then calculated using eq. 2.

$$\text{Oleoresin yield (\%)} \frac{\text{g}}{\text{g}} \times 100 = \frac{W_a - W_b}{W_s} \times 100 \quad (2)$$

where, W_a is weight of the round bottom flask after purification process in gram, W_b is weight of the round bottom flask before purification process in gram and W_s is raw sample weight is gram. Experiment was conducted triplicate.

2.3 Hot compressed water extraction (HCWE)

Hot compressed water extraction (HCWE) was carried out using an Accelerated Solvent Extractor (ASE 200) (Dionex Ltd., Camberley, Surrey, UK). The schematic diagram of the ASE 200 is shown in **Fig. 1**.

The system comprises an extraction cell, solvent storage, collection vial, pump, and two valves: a purge valve and a static valve. Prior to extraction, the ASE 200 system was purged with nitrogen gas by opening the purge valve. The oven was then preheated to the required temperature, and once the set temperature was reached, the extraction cell was automatically transferred into the oven. Distilled water from the storage reservoir was pumped into the extraction cell, which had been preloaded with 3 ± 0.01 g of dried ginger. The extract was subsequently transferred to the collection vial via the static valve, while nitrogen gas (N_2) was used to flush any residual extract.

In this study, the extraction temperature was varied from 130 to 200°C, while the extraction pressure and extraction time were kept constant at 3.5 MPa and 30 minutes, respectively. Other process parameters were standardized as follows: flush volume of 60 %, purge volume of 60 seconds, static cycle of 1, and heating time of 7 minutes.

For yield calculation, the water was removed from the extract using a rotary evaporator (Buchi R-205, Switzerland) under vacuum pressure (72 mBar), with a water bath temperature of 50°C, condenser temperature of 10°C, and a rotation speed of 120 rpm. The yield percentage was determined using Equation 2. Experiment was conducted triplicate.

2.4 Ginger bioactive compound analysis method

Ginger bioactive compound analysis method was performed using High-Performance Liquid Chromatography (HPLC) (Waters Corp., MA, USA) equipped with a Photodiode Array (PDA) detector and a Lichrocart 250-4,6 Purospher Star RP-8E (5 µm) column (MERCK, Germany). The total run time was set to 10 minutes, with an injection delay of 8 minutes. Two mobile phases were prepared: Solution A, consisting of 100 % acetonitrile, and Solution B, comprising 65 % (v/v) methanol in water. The chromatographic separation was performed under a linear elution, in which the mobile phase composition was progressively changed from 20:80 (A:B, v/v) to 50:50 (A:B, v/v) at a constant flow rate of 1.20 mL/min. Prior to injection, sample extracts (10 µL) were filtered through a 0.45 µm PTFE syringe filter (Whatman, USA) to remove particulates. The full analytical procedure has been described in detail by Md Sarip et al. (2024)²³.

Table 1 Sample description for ginger sample.

Sample name	Range size (mm)	Sieve ASTM E-11 code
Large	3.36 to 2.00	No.6 to No.10
Medium	2.00 to 1.00	No.10 to No.18
Small	1.00 to 0.50	No.18 to No. 35

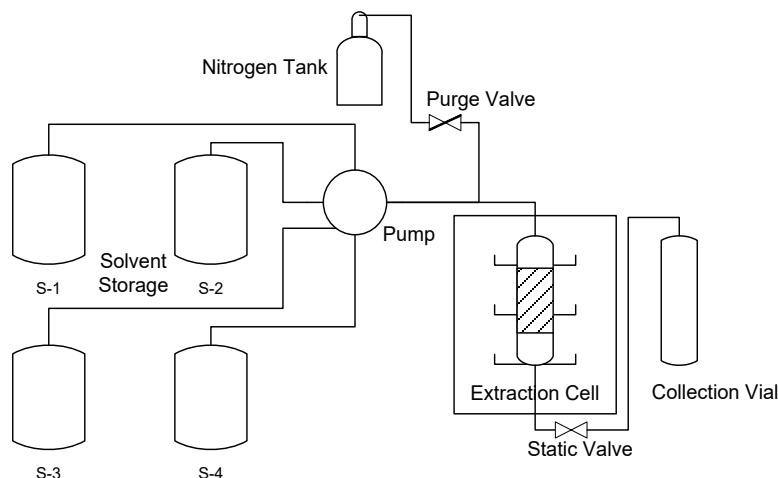


Fig. 1 The schematic diagram for ASE 200.

3 Results

3.1 Screening process on the effect of particle size on the ginger oleoresin and oil yield

To ensure that the focus of the hot compressed water extraction (HCWE) study remains on process parameters rather than raw material variability, a preliminary screening was conducted to evaluate the effect of particle size on ginger oleoresin and oil yields. This step is essential to determine whether particle size significantly influences extraction efficiency, thereby allowing it to be excluded as a variable in subsequent experiments. The effect of the particle size on the ginger oleoresin and oil yield is shown in **Table 2**.

As shown in **Table 2**, the small sample size exhibited the lowest yield percentage at 6.77 ± 0.22 %, followed by the medium sample at 7.48 ± 0.14 %, and the large sample at 7.57 ± 0.31 %. Similar trends are observed from 3.88 ± 0.18 % to 4.38 ± 0.97 % for small to large particle size in ginger oil extraction. This trend contradicts the theoretical expectation that smaller particle sizes should yield higher extraction efficiency due to their larger surface area, which enhances mass transfer. However, this deviation can be attributed to higher volatilization losses of pungent compounds during sample preparation, particularly during drying stage²⁴. The increased surface area of smaller particles accelerates undesirable mass transfer, leading to greater losses of volatile bioactive compounds²⁵. Additionally, sample preparation techniques such as grinding, drying, and exposure to heat are known to reduce the concentration of heat-sensitive and volatile compounds in plant matrices²⁶. But the difference in yield between large and small samples is insignificant with *p*-value of 0.933 and 0.77 for oleoresin and oil yield, respectively. This is suggesting that within the selected size range from 0.5 to 3.36 mm, ginger particle size did not significantly influence the ginger oleoresin and oil mass transfer process. Similar findings were reported by Eikani et al. (2007)²⁷, who observed no significant difference in extraction efficiency for cumin particle sizes between 0.25 cm and 0.5 cm. **Table 2** also shows the physical properties of the oleoresin and oil. In this study, different particle sizes did not affect the odor and color characteristics of either the oleoresin or the oil product. Ginger oleoresin obtained from solvent extraction is a black, viscous oil with a warm, spicy odor, believed to originate from the pungency of its bioactive compounds, such as gingerol and shogaol. Meanwhile, ginger oil appears yellowish in color with a lemony-balsamic odor. Therefore, ground and dried ginger particles within this size range were not separated and were used collectively in subsequent experimental work.

3.2 Comparison between hot compressed water extraction (HCWE) and other extraction method

The HCWE was performed at a constant pressure of 3.5 MPa and a fixed temperature of 140 °C for 30 minutes extraction time. The physical appearance of the resulting ginger extract, as observed post-extraction, is illustrated in **Fig. 2**.

As shown in **Fig. 2(c)**, HCWE ginger extract exhibits physical characteristics like those of ginger oleoresin (**Fig. 2(a)**) obtained through traditional solvent extraction, but it is less oily, likely due to a lower wax content. It presents as a thick, crystalline, resinous black solid. This texture and appearance are indicative of a highly concentrated extract rich in pungent phytochemicals such as 6-gingerol and 6-shogaol, and related compounds. Additionally, the extract emits a light, warm, spicy, and mildly pungent aroma, typical of volatile constituents found in ginger. The dark coloration of the extract suggests a high concentration of thermally stable bioactive compounds, possibly resulting from Maillard reactions, oxidation, or partial caramelization during the HCWE process. These chemical changes are known to influence both the

sensory profile and chemical composition of ginger extracts²⁴). Meanwhile, the extract was distinctly different from ginger oil produced via hydro distillation.

Given its similarity to ginger oleoresin in both physical form and aromatic profile, the HCWE extract was further analyzed based on oleoresin compositional analysis, including quantification of key constituents such as gingerol and shogaol derivatives. This compositional profiling is essential to evaluate its pharmacological potential and suitability as a natural therapeutic or functional food additive. A comparison of the chemical composition of HCWE ginger extract and ginger oleoresin is presented in **Table 3**.

As shown in **Table 3**, HCWE achieved a higher extraction yield of 10.37 ± 0.33 % (g/g) for 30 minutes compared to solvent extraction of 7.57 ± 0.31 % (g/g) for 480 minutes. This indicating enhanced extraction efficiency under subcritical conditions through enhancing mass transfer and disrupted plant matrix integrity, resulting in improved solubilization of water-soluble components¹⁹). Despite the higher yield, HCWE yielded significantly lower concentrations of 6-gingerol at 1957.22 ± 2.55 µg/g compared to solvent extraction at 8406.99 ± 3.98 µg/g. Notably, 6-shogaol was not detected in the HCWE extract showing the selective extraction, while solvent extraction recovered a substantial concentration of 716.76 ± 9.06 µg/g. This difference can be attributed to the thermal degradation pathways of gingerols and shogaols. Solvent extraction using ethanol at moderate temperatures preserved both compounds, whereas the subcritical water environment of HCWE may have promoted the degradation or transformation of shogaols and the partial degradation of gingerols. This transformation has been reported by previous researchers, where 6-gingerol was converted into 6-shogaol through heat-related processes, including hot compressed water extraction^{19,28}) and puffing²⁹).

In conclusion, the HCWE ginger extract closely resembles ginger oleoresin in appearance and aroma but is less oily, suggesting reduced wax content. While HCWE achieved a higher yield, it produced significantly lower concentrations of 6-gingerol and no detectable 6-shogaol compared to solvent extraction. This outcome is referred to the selective extraction as features in HCWE process. HCWE offers a selective and environmentally friendly fast extraction method with potential applications that require highly pure bioactive compounds and reduced solvent usage. Further optimization is necessary to enhance the recovery of key bioactive compounds for therapeutic or functional food development.

Table 2 The effect of particles size on the ginger oleoresin and oil yield, and physical appearance of the product.

Extraction method	Parameter	Particle sizes			Physical appearance	
		Small	Medium	Large	Color	Odor
Solvent extraction	Oleoresin yield, % (w/w)	6.77 ± 0.22	7.48 ± 0.14	7.57 ± 0.31	Black viscous oil color	Balsamic warm and Spicy
Hydro distillation	Oil yield, mL/g	3.89 ± 0.20	4.76 ± 0.84	4.39 ± 0.98	Yellowish oil color	Lemony-bal-samic

Table 3 Comparison between solvent extraction and HCWE process at 140°C and 3.5 Mpa.

Extraction method	Product	Yield (g/g)	6-Gingerol Concentration (µg/g)	6-Shagaol Concentration (µg/g)
Solvent extraction	Ginger oleoresin	7.57 ± 0.31	8406.99 ± 3.98	716.76 ± 9.06
HCWE process	HCWE ginger extract	10.37 ± 0.33	1957.22 ± 2.55	0

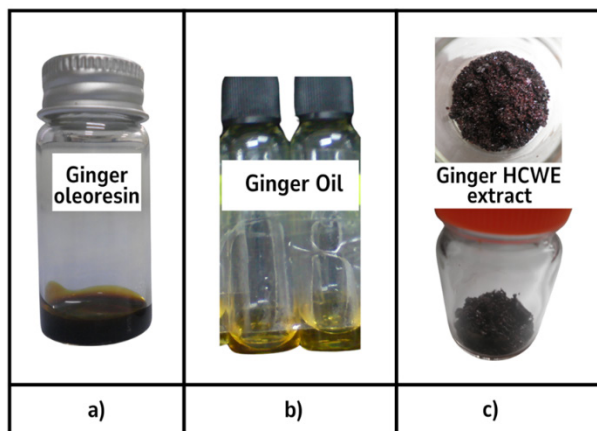


Fig. 2 The physical appearance of ginger product. a) Ginger oleoresin b) Ginger oil d) Ginger HCWE extract.

3.3 Effect of temperature in hot compressed water extraction

Effect of the extraction temperature on each ginger bioactive compound concentration at constant pressure of 3.5 MPa and 30 minutes of time is shown in **Fig. 3**.

At constant pressure and time, the concentration profile for 6-gingerol and 6-Shogaol can be differentiate into 3 regions. In 1st region (gingerol dominated) from 130 to 140°C, the concentration 6-gingerol increased from $1741.54 \pm 0.96 \mu\text{g/g}$ to $1957.22 \pm 2.55 \mu\text{g/g}$. In this region, no concentration of 6-shogaol and 10-gingerol was observed. Then, the concentration of the gingerol decrease to $851.95 \pm 2.34 \mu\text{g/g}$ at the temperature of 170°C, and the concentration of 6-shogaol increase to $1135.23 \pm 1.18 \mu\text{g/g}$ at the same temperature for the 2nd region (shogaol dominated). In this region, the dehydration of 6-gingerol into 6-shogaol is observed to occur as discover by previous study^{19, 28, 29}.

Concentration pattern for both compounds can be clearly seen in the HPLC chromatogram where the 6-gingerol peak slowly decrease as temperature increase and 6-shogaol peak are increase starting at the temperature of 140°C as illustrated in **Fig. 4**. In the 3rd region (degradation dominated), both components: 6-gingerol and 6-shogaol start to decrease from the temperature 170 to 200°C. This showed the rapid degradation of ginger oleoresin occurred as evidence from the production of burning smell and present of the carbon in the extract as shown in **Fig. 5(b)**. This phenomenon as supported by the rapid increased of oleoresin yield up to 4-fold as shown in **Fig. 5(a)**.

At the temperature of 130 to 160°C, ginger HCWE yield percentage slightly increase from 9.67 to 12.21 % (w/w). From 160 to 200°C, the ginger HCWE yield was rapidly increase from 12.21 to 44.70 ± 0.33 % (g/g). At lower temperature below 160°C, the extraction mainly contributed to mass transfer process where the solute transferred from the matric fiber. Above 160°C, the degradation reaction process occurred with the mass transfer process, where some of the solute and fiber degraded to the carbon-based product which contribute to significant increase of yield to up 4-fold and concentration of bioactive are significantly loses to form the unwanted product. This supported on the possible unintended degradation occur during the extraction process above 160°C.

Degradation of solutes in HCWE was anticipated, as reviewed by Cheng et al. (2021), who reported that degradation can begin at temperatures as low as 150 °C, depending on the solute characteristics. Further experiments are required to identify the precise degradation temperature³⁰. Therefore, based on this study, it is suggested that the HCWE process should not be conducted at higher temperatures (160 °C and above) for ginger extraction.

Although the HCWE process produces a lower concentration of bioactive compounds, it offers selective extraction, as shown in Figure 4. The HPLC chromatogram of the solvent extraction process demonstrates that all components are extracted using this method, as illustrated in Figure 6.

As shown in **Fig. 6**, a broad spectrum of extracted compounds, indicating a non-selective process of the solvent extraction process. While this approach ensures maximum recovery of both major and minor constituents, it may also co-extract unwanted or thermally sensitive compounds, potentially complicating downstream processing. The distinct chromatographic profiles highlight a fundamental difference between the two methods: HCWE tends to favor the extraction of thermally stable and water-soluble bioactive under subcritical conditions, while solvent extraction extracts a wider range of compounds due to the broader solvating capacity of ethanol. This comparative insight supports the potential of HCWE as a greener and more time-efficient alternative for extracting specific ginger bioactive, owing to its reduced reliance on organic solvents and shorter processing times. This makes it particularly valuable in formulations where

purity and specificity are critical. It's also noted that, other ginger bioactive compound that is 10-gingerol exist at a much lower concentration of $39.67 \pm 5.46 \mu\text{g/g}$ at the temperature of 170°C in HCWE process.

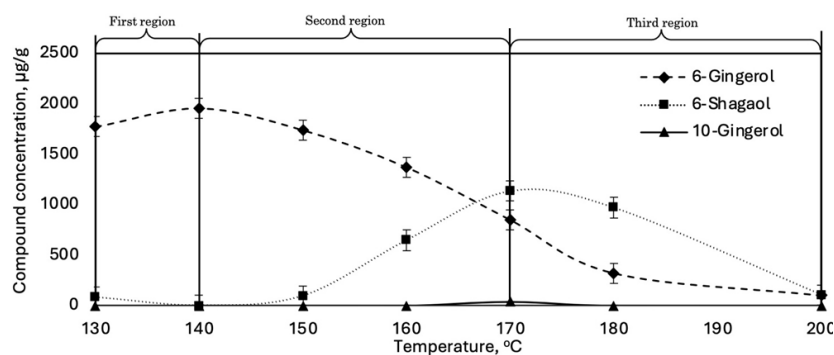


Fig. 3 The effect of temperature in HCWE process to the ginger bioactive compounds concentration, $\mu\text{g/g}$ at constants pressure (3.5 MPa), time (30 minutes) and solvent to ratio (28:3 mL/g).

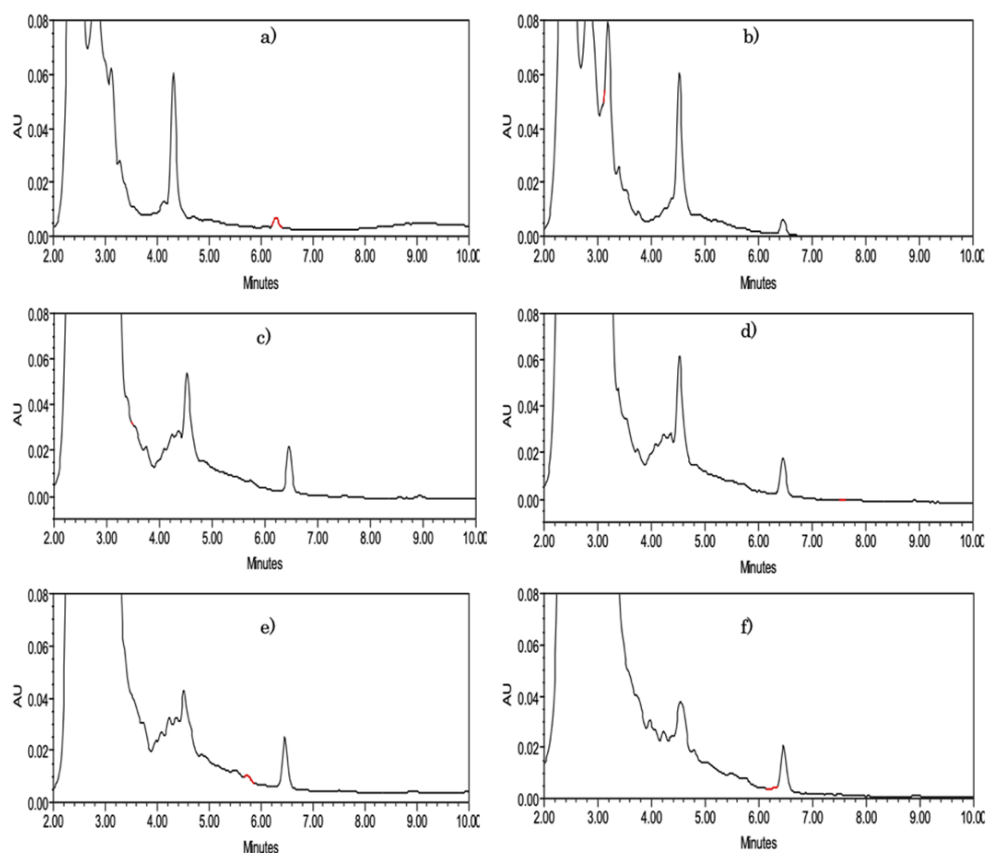


Fig. 4 HPLC profile for HCWE extract at different temperature; a) 130°C ; b) 140°C ; c) 150°C ; d) 160°C ; e) 170°C and f) 180°C at constants pressure (3.5 MPa), time (30 minutes) and solvent to sample ratio (28:3 mL/g).

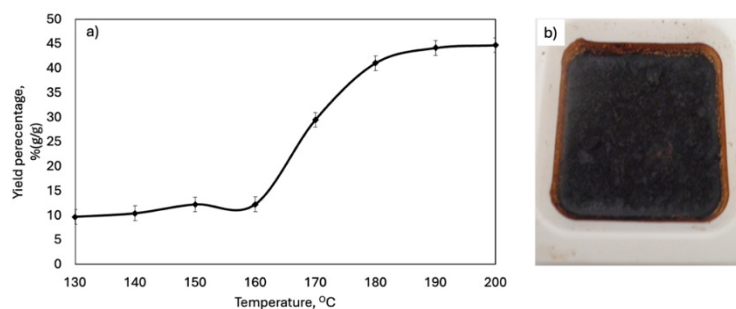


Fig. 5 Effect of temperature on the ginger yield percentage. b) Ginger HCWE extract at 200 °C.

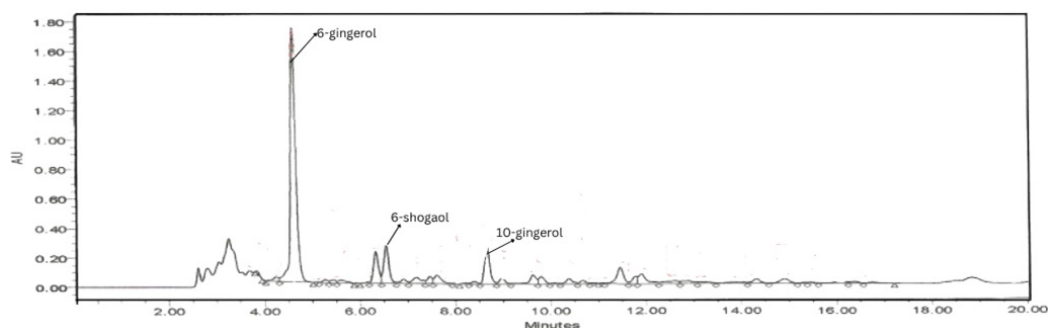


Fig. 6 HPLC profile for solvent extraction.

4 Conclusions

As a conclusion, initial screening of particle size revealed minimal impact on extraction yield or product quality, allowing standardized particle sizes to be used across all experiments. Compared to solvent extraction and hydrodistillation, HCWE yielded a product that was physically and organoleptically similar to ginger oleoresin, but notably less oily, likely due to lower wax content. HCWE also resulted in a higher extraction yield of 10.37 ± 0.33 % (g/g) than solvent extraction at 7.57 ± 0.31 % (g/g), showcasing its enhanced extraction efficiency under subcritical conditions. However, HCWE produced significantly lower concentrations of 6-gingerol at 1957.22 ± 2.55 µg/g and 0 concentration of 6-shogaol, in contrast to the ethanol-based solvent extraction which yielded of 8406.99 ± 3.98 µg/g of 6-gingerol and 716.76 ± 9.06 µg/g of 6-shogaol. These findings highlight the selective process of HCWE. Bioactive compound concentration showed a temperature-sensitive profile with three distinct regions: a gingerol-dominated region (130–140°C), a shogaol-dominated region (150–170°C), and a degradation-dominated region beyond 170°C. The transition of 6-gingerol to 6-shogaol at mid-range temperatures confirmed thermal transformation pathways previously reported in the literature. However, above 160°C, rapid degradation was observed, with significant bioactive loss and increased extraction yield attributed to the formation of unwanted carbonaceous by-products. Therefore, temperatures above 160°C are unsuitable for bioactive-targeted extraction due to the risk of compound degradation, although they may improve overall mass recovery. HPLC chromatographic comparisons further revealed that HCWE leads to a more selective profile, extracting fewer but more stable compounds compared to solvent extraction, which retrieves a broader array of compounds, including thermally sensitive ones. This selectivity may be advantageous for applications demanding high purity, minimal solvent use, and greener processing conditions. In summary, HCWE is a promising, sustainable extraction technique capable of producing ginger extracts with desirable physical and sensory qualities and acceptable bioactive content under controlled temperature conditions. While its selectivity may limit the range of extracted compounds, it offers a fast and cleaner alternative with lower environmental impact. Future work should focus on optimizing HCWE parameters, particularly temperature and residence time to balance yield and bioactive preservation, and to explore potential integration with other green technologies for enhanced recovery and application in pharmaceutical or nutraceutical formulations.

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