Heliyon 9 (2023) e16516

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

# Evaluation of antioxidant capacity, structure, and surface morphology of ginger (*Zingiber officinale*) using different extraction methods

Paola Jorge-Montalvo [a,*], Carlos Vílchez-Perales [b], Lizardo Visitación-Figueroa [a]

[a] Center for Research in Chemistry, Toxicology and Environmental Biotechnology, Department of Chemistry, Faculty of Science, Universidad Nacional Agraria La Molina, 15024, Lima, Peru
[b] Department of Nutrition, Faculty of Zootechnic, Universidad Nacional Agraria La Molina, 15024, Lima, Peru

ARTICLE INFO

*Keywords:*
Relative polarity
Solubility
Bioactive compounds
Thermal stress

ABSTRACT

The antioxidant capacity of ginger depends on the type of variety, growing conditions, post-harvest, drying method, extraction, and measurement, among others. The objective of the research was to compare the efficiency of ultrasound (US), magnetic agitation (AM), maceration (M), and reflux (R) extraction methods. In the GFD (fresh air-dried ginger) extract, the contents of total phenolic content (TFC), 6-gingerol (6-G), and 6-shogaol (6-S) were evaluated; as well as the antioxidant capacity by FRAP (ferric reducing antioxidant power) and $IC_{50}$ in DPPH (2,2-diphenyl-1-picrylhydrazyl radical). In addition, structural and morphological changes were evaluated with FTIR and SEM, respectively. The results for all extraction methods for TFC, 6-G, and 6-S were between 9.422 and 10.037 mg EAG/g dry matter (dm), 4.072–4.838, and 0.194–0.263 mg/g dm respectively, with the highest values for TFC and 6-G for M and 6-S for R. FRAP and $IC_{50}$ in DPPH values were between 0.172 and 0.192 mmol $Fe^{2+}$/g dm and 0.531–0.722 mg dm/mL respectively, presenting higher antioxidant capacity in M and R1, R2, and R3 (1, 2, and 5 h, respectively). Extracts from M and R1 methods presented lower FTIR transmittance values and greater changes in their surface morphology, with folds and breaks in the starch granules by SEM images. It is concluded that extracts with higher antioxidant capacity are obtained using medium polarity solvents such as methanol and with M and R1 methods. Because of the longer extraction time and moderate thermal stress, the structure and surface morphology of the extracted GFD sample showed greater changes on the surface of the starch granules and, consequently, greater extraction of bioactive compounds.

## 1. Introduction

Extraction methods of bioactive compounds from ginger rhizome use different solvents, extraction equipment, temperature, and time conditions, which influence the efficiency of the extraction method of bioactive compounds with an antioxidant capacity [1–3]. In the extraction process of bioactive compounds, current methods such as ultrasound, and supercritical fluids, among others, are used, leaving aside traditional methods such as maceration, agitation, Soxhlet, and reflux systems; due to a lower use of solvents, extraction time and energy requirement; although not necessarily the current methods are more efficient since it depends on the matrix studied.

* Corresponding author.
*E-mail address:* paolajom@lamolina.edu.pe (P. Jorge-Montalvo).

https://doi.org/10.1016/j.heliyon.2023.e16516
Received 14 December 2022; Received in revised form 13 May 2023; Accepted 18 May 2023
Available online 23 May 2023

The temperature and time in the extraction process have been evaluated by several authors finding differences in the antioxidant capacity. Yeh et al. [2] obtained better results of antioxidant capacity when using ethanol at 25 °C, compared to hot water using the reflux method. Si et al. [4] determined a high antioxidant capacity when the temperature was between 60 and 90 °C with a time less than 8 h using stirring. Prasanna et al. [5] determined that the antioxidant capacity of ginger methanolic extract obtained by ultrasound decreases with increasing temperature from 30 to 45 °C. Ok & Jeong [6] determined that an ethanolic extraction with the reflux method at 80 °C for 24 h decreases the concentration of 6-G and increases the concentration of 6-S due to the degradation of 6-G and formation of 6-S, similar to what occurs when drying at higher temperature is used. On the other hand, 6-S presented higher antioxidant capacity than its predecessor, 6-G, the latter being transformed with increasing temperature and in the presence of an acid catalyst [7]. Ko et al. [8] were able to obtain a higher content of 6-S during the extraction process with subcritical water at 190 °C for 15 min, they concluded that the higher the temperature and time, the higher the transformation of 6-G to 6-S is achieved. Likewise, Ghasemzadeh et al. [9] obtained a similar result by applying the reflux method at 80 °C for 4 h and indicated that the 6-S content depends more on the temperature than on the extraction time. Jelled et al. [10] for methanol extracts at 25 °C determined that the antioxidant capacity decreases due to temperature effects and handling as a powder, due to the degradation of phenols during processing. Li et al. [3] used methanol extraction and ultrasound for 30 min and obtained higher antioxidant capacity for dried samples compared to charred, sautéed, and fresh samples. Griškevičienė et al. [11] using the reflux method at 90 °C obtained higher extraction of phenolic compounds content in 1.5 h compared to 3 h extraction time; this is due to the rapid solubilization of gingerols in the solvent and subsequent thermal degradation at prolonged times [12]. Therefore, in the present investigation, the effect of time and temperature on extraction efficiency was studied by comparing traditional methods with current methods.

The study of structural changes by FTIR determines the interactions at the intermolecular level that change in ginger with the extraction processes, FTIR spectra show absorption bands corresponding to cellulose, hemicellulose, lignin, and phenolic compounds [13]. The morphological changes of the surface of ginger rhizome by SEM subjected to different extraction methods were studied by Liu et al. [14], they found the formation of folds and small breaks as forms of alteration of the surface of the starch granules of ginger rhizome.

Therefore, the research work aims to compare the efficiency of the extraction methods by ultrasound, magnetic stirring, maceration, and reflux (1, 2, 5, 8, and 12 h) through the content of bioactive compounds, antioxidant capacity, and changes in the structure and surface morphology of fresh air-dried ginger.

## 2. Materials and methods

### 2.1. Materials

The fresh samples of ginger rhizome *Zingiber officinale* Roscoe of Creole variety were collected at the post-harvest plant Elisur Organic S.A.C. located in Pichanaki, Junín (21.6 °C minimum temperature, 33.8 °C maximum temperature, 75.7% humidity and 3.65 mm/day of precipitation), the main place of ginger production in Peru. The collection was performed randomly on March 22 and 24, 2021, taking subsamples from each lot, and for the sample, size reduction was homogenized using the coning and quartering technique [15]. Folin-Ciocalteu, 2,4,6-tripyridyl-*s*-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), L-ascorbic acid, gallic acid reagents were purchased from Sigma-Aldrich (MO, USA). Standards of 6-gingerol (purity ≥97%) and 6-shogaol (purity ≥99.9%) were purchased from TRC (ON, CAN) and ChromaDex (CA, USA) respectively. Methanol and acetonitrile, HPLC grade, were purchased from J.T. Baker (NJ, USA).

### 2.2. Proximal analysis and sample treatment

Proximate analysis of fresh ginger was performed using methods AOAC-930.04 for moisture, AOAC-930.05 for ash, AOAC-978.04 for total protein, AOAC-930.09 for ethereal extract, AOAC-962.09 for crude fiber, the nitrogen-free extract was obtained by difference. The method proposed by Eleazu & Eleazu [16] was used for the determination of oleoresin content.

For the extraction process and analysis of bioactive compounds, antioxidant capacity, and surface and structural morphology, fresh ginger was washed, cut into 3 mm thick slices, dried under shade, and natural aeration until constant weight at an average temperature of 25 °C (GFD). According to Sangwan et al. [17] this drying method maintains the organoleptic, nutritional, and phenolic compound properties of ginger. Although, Ok & Jeong [6] indicate that there is a variation of 6-gingerol content of less than 18% and 6-shogaol of less than 3% when comparing freeze-drying and natural aeration drying methods. Finally, the GFD sample was crushed in a mill, sieved with a 0.25 mm mesh, and stored at 4 °C until further extraction and analysis.

### 2.3. Extraction process

The GFD sample was subjected to four extraction methods: ultrasound (US), magnetic stirring (AM), maceration (M), and reflux (R). The US method was performed with an ultrasonic processor (VC505, Sonics & Materials INC, CT, USA), with 40% amplitude, for the evaluation of the influence of the solvent, different concentrations of 70, 80 and 100% (v/v) of methanol were used, with a load of 6.25 mg/mL at 40 °C for 20 min with a single extraction; for the comparison with the other extraction methods, a load of 6.25 mg/mL at 40 °C with two extractions of 20 min was used. In AM method with methanol, the magnetic stirrer (M6.1, CAT, DEU) was used, at 400 rpm, with a load of 3.125 mg/mL at 25 °C for 40 min. In the M method with methanol, a load of 3.125 mg/mL at 25 °C for 24 h was used. In R method with methanol, the fat and oil extractor (FA-46, MRC, ISR) was used, with a load of 3.125 mg/mL at 85 °C for 1, 2, 5,

8, and 12 h.

### 2.4. Determination of bioactive compounds

The total phenols method reported by Singleton et al. [18] was used. Briefly, to 50 μL of the GFD extract, 1 mL of fresh Folin Ciocalteau solution (diluted 10-fold) was added followed by 950 mL of 7.5% (w/v) $Na_2CO_3$. The gallic acid calibration curve was prepared from 0.5 to 12.5 μg/mL, proceeding similarly to the extract. The reaction time was 15 min at 45 °C, readings were measured at 765 nm in a spectrophotometer (Evolution 300, Thermo Fisher Scientific, WI, USA). Total phenolic content (TFC) was expressed as milligrams of gallic acid equivalent per gram of dry matter (mg GAE/g dm).

The determination of 6-G and 6-S was performed as proposed by Vipin et al. [1], using an HPLC system (Ultimate 3000, diode array detector, Thermo Scientific, WI, USA), column (250 mm × 4.6 mm x 5 μm, Supelco Purospher® STAR, DEU), and column guard (4.6 mm, Phenomenex, CA, USA). Briefly, 20 μL of the extracted GFD or standard was injected at a flow rate of 1 mL/min at 25 °C; with an acetonitrile gradient of 5–50% from 0 to 8 min, 50–65% from 8 to 17 min, 65–100% from 17 to 38 min, and 100 to 45% from 38 to 45 min. Absorbance readings were taken at 230 nm. Calibration curves for 6-G and 6-S ranged from 5.7 to 85.6 μg/mL and from 1.2 to 17.6 μg/mL, respectively. The results were expressed in mg/g dm.

### 2.5. Determination of antioxidant capacity

The ferric reducing antioxidant power (FRAP) method according to Benzie & Strain [19] was used. The FRAP reagent was prepared in a 10:1:1 ratio with the reagents 0.3 M acetate buffer at pH 3.6, 10 mM ferric tripyridyl triazine in HCl 40 mM, and 20 mM ferric chloride hexahydrate. Briefly, 50 μL of the GFD extract was dissolved in 1 mL volume; then, 1 mL of a fresh solution of FRAP reagent was added, with a reaction time of 15 min at 37 °C in the dark. Readings were taken at 593 nm using the same spectrophotometer described above. The calibration curve was prepared using standard solutions of $FeSO_4$ at concentrations of 2.5–25 μM $Fe^{2+}$ and proceeded similarly to the extract.

The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) uptake method according to Mensor et al. [20] with modifications was used. Dilutions of the GFD extract were prepared from 50 to 200 μL to a volume of 1 mL, and 0.5 mL of fresh DPPH solution was added. The reaction time was 30 min in the dark at room temperature. Readings were taken at 518 nm using the same spectrophotometer described above. The results are expressed as percentage inhibition ($IC_{50}$) in mg dm/mL.

### 2.6. Analysis of the structure and surface morphology

The GFD sample without extraction and with extraction by US, AM, M, and R methods was brought to dryness with silica gel and under vacuum for 48 h. For the analysis of the changes in the chemical structure of the samples, a Fourier transform infrared spectrophotometer FTIR (Nicolet iS10, Thermo Fisher Scientific, WI, USA) was used, with a spectrum between 700 and 3500 $cm^{-1}$ [21]. For the analysis of the changes in the surface morphology of the samples, a scanning electron microscope SEM (Q250 Analytical, Thermo Fisher Scientific, WI, USA) was used, where the samples were covered with a gold film, with a voltage acceleration of 20 kV and 2400 magnification [13,21]. In addition, fresh ginger was imaged using an optical microscope (MB1152, MicroBlue Bino, Euromex, NLD) at S400× magnification.

### 2.7. Statistical analysis

The estimation of the results of bioactive compounds (TFC, 6-G, and 6-S) and antioxidant capacity (FRAP and $IC_{50}$ in DPPH) for each extraction method were performed in triplicate, reporting the mean value and its standard deviation. Differences were evaluated by one-way analysis of variance (ANOVA) followed by Duncan's multiple test using XLSTAT software version 2021.5.1. (Addinsoft, NY, USA). Significance was accepted at $p < 0.05$.

**Table 1**
Results of proximate analysis of fresh ginger. Data represent mean ± standard deviation, n = 3.

| Parameters | Content g/100 g | According to reference g/100 g |
|---|---|---|
| Ash | 4.20 ± 0.01 | 3.30–13.10 |
| Crude fiber | 5.10 ± 0.08 | 0.92–21.90 |
| Total protein | 6.50 ± 0.03 | 1.80–12.05 |
| Ether extract | 0.50 ± 0.01 | 0.76–17.11 |
| Nitrogen-free extract | 67.1 ± 0.06 | 39.70–71.46 |
| Oleoresin | 4.64 ± 0.18 | 2.93–8.6 |

## 3. Results and discussion

### 3.1. Proximal analysis

The results of the proximate analysis are presented in Table 1. The ash, crude fiber, total protein, ethereal extract, and nitrogen-free extract contents of the fresh sample were within the expected values [17,22–24]; and the amount of oleoresin was slightly lower than the values reported by Eleazu & Eleazu [16]. The concentrations of chemical components and oleoresin content depend on the variety, state of the fresh or dry sample, soil quality, agroclimatic conditions, place of origin, maturity stages, and adaptive metabolism of the plant, among others [25,26].

### 3.2. Bioactive compounds

The comparative analysis of the different methods of extraction of total phenols, 6-G and 6-S from the GFD sample are shown in Table 2. The results of the extraction by method M presented higher TFC and 6-G values that differed significantly ($p < 0.05$) from the rest of the extractions with other methods, and the 6-S content in the extract obtained by method R was significantly higher ($p < 0.05$) compared to the other extraction methods.

The highest extraction efficiency of TFC was presented by method M (10.037 ± 0.140 mg/g dm.), this method is recommended when the bioactive compounds are thermolabile [11,14]. On the other hand, methods R1 (9.422 ± 0.327 mg/g dm), R2 (9.654 ± 0.603 mg/g dm), R3 (9.696 ± 0.168 mg/g dm), R4 (9.790 ± 0.323 mg/g dm), R5 (9.487 ± 0.354 mg/g dm), US (9.823 ± 0.221 mg/g dm), and AM (9.986 ± 0, 156 mg/g dm) showed no significant differences despite being subjected to different extraction times and temperatures that may influence the solubility and transformation of phenols by dehydration, retro-aldol, and reduction mechanism, among others [27].

The highest 6-G extraction efficiency is observed with the M method (4.838 ± 0.049 mg/g dm) at 24 h compared to the AM method (4.689 ± 0.078 mg/g dm) at 40 min, both at 25 °C, because a prolonged exposure (24 h) of the GFD to the solvent has a larger exposed surface and therefore a higher diffusion of 6-G, according to the second Fick's Law. On the other hand, if the M time is shorter, as well as the AM time, a better result is obtained for AM, as reported by Liu et al. [14]. Furthermore, it is observed that comparing the method M and R1 (4.613 ± 0.191 mg/g dm), R2 (4.646 ± 0.136 mg/g dm), and R3 (4.535 ± 0.019 mg/g dm) there is a decrease in 6-G content, because the temperature increase favors the dehydration of 6-G consisting in the loss of the β-hydroxy keto group from the alkyl tail and formation of a double bond, generating a new more stable structure, 6-S [7]. Thus, the lowest extraction efficiency occurs with method R4 (4.072 ± 0.139 mg/g dm) and R5 (4.126 ± 0.166 mg/g dm) due to the high temperature and very long times; under these conditions, 6-G dehydrates or undergoes retro-aldol reactions reducing its concentration [14,27]. The US method (4.547 ± 0.101 mg/g dm) presented similar extraction efficiency to methods R1, R2, and R3, with different extraction times and temperatures, because the effects of acoustic cavitation [14,28] were not very efficient in breaking the cell wall and exposing phenolic compounds such as 6-G to methanol.

In 6-S extraction, the highest efficiency was presented by method R1 (0.2576 ± 0.013 mg/g dm), R2 (0.263 ± 0.010 mg/g dm), R3 (0.254 ± 0.011 mg/g dm), R4 (0.245 ± 0.019 mg/g dm) and R5 (0.242 ± 0.042 mg/g dm) compared to AM (0.209 ± 0.002 mg/g dm) and M (0.210 ± 0.001 mg/g dm) methods, due to the dehydration of 6-G by the effect of higher temperature, similar results were reported by Ok & Jeong [6]. When working with the same temperature conditions as the M and AM method both at 25 °C and R1, R2, R3, R4, and R5 at 85 °C, no significant variation of 6-S content was obtained, suggesting some stability of 6-S over time. The 6-S by presenting a double bond product of the dehydration of the β-hydroxy keto group of 6-G presents a lower polarity, so its solubility is higher in methanol at 85 °C compared to methanol at 25 °C, due to the reduction of the dielectric constant from 33.3 to 28 of methanol. The least efficient extraction was by the US method (0.194 ± 0.005 mg/g dm), due to the low efficiency of cavitation to break the plant cell wall, as reported for 6-G.

**Table 2**
Comparison of extraction methods on the content of bioactive compounds of fresh air-dried ginger (GFD). Data represent mean ± standard deviation, n = 3.

| Extraction method | Time | Temperature °C | Total Phenolics[1] | 6-Gingerol | 6-Shogaol |
|---|---|---|---|---|---|
| | | | (mg/g dry matter) | | |
| US | 40 min | 40 | 9.823 ± 0.221 $^{ab}$ | 4.547 ± 0.101 $^{b}$ | 0.194 ± 0.005 $^{a}$ |
| AM | 40 min | 25 | 9.986 ± 0.156 $^{ab}$ | 4.689 ± 0.078 $^{c}$ | 0.209 ± 0.002 $^{b}$ |
| M | 24 h | 25 | 10.037 ± 0.140 $^{b}$ | 4.838 ± 0.049 $^{d}$ | 0.210 ± 0.001 $^{b}$ |
| R1 | 1 h | 85 | 9.422 ± 0.327 $^{a}$ | 4.613 ± 0.191 $^{bc}$ | 0.258 ± 0.013 $^{cd}$ |
| R2 | 2 h | 85 | 9.654 ± 0.603 $^{ab}$ | 4.646 ± 0.136 $^{bc}$ | 0.263 ± 0.010 $^{d}$ |
| R3 | 5 h | 85 | 9.696 ± 0.168 $^{ab}$ | 4.535 ± 0.019 $^{b}$ | 0.254 ± 0.011 $^{cd}$ |
| R4 | 8 h | 85 | 9.790 ± 0.323 $^{ab}$ | 4.072 ± 0.139 $^{a}$ | 0.245 ± 0.019 $^{cd}$ |
| R5 | 12 h | 85 | 9.487 ± 0.354 $^{ab}$ | 4.126 ± 0.166 $^{a}$ | 0.242 ± 0.042 $^{c}$ |

[1] Gallic acid equivalent. Different letters in the same column mean statistically different ($p < 0.05$) by Duncan's multiple range test.

### 3.3. Antioxidant capacity

The results of antioxidant capacity evaluated by FRAP and by $IC_{50}$ in DPPH presented the following trend M > R1>R2>R3>AM > US > R4>R5 as shown in Table 3. The extractions methods M, R1, R2, and R3 presented higher antioxidant activity which differed significantly ($p < 0.05$) from the other extraction methods, due to the exposure time of up to 5 h with the solvent; which favors the dissolution of metabolites with antioxidant capacity such as TFC, 6-G, and 6-S [2,5,24,26], being the 6-S of higher antioxidant capacity due to presenting the double bond in the alkyl tail [8]; however at prolonged times as in the case of R4 and R5, a significant decrease ($p < 0.05$) of the antioxidant capacity due to the degradation of phenols by dehydration and the retro-aldol reaction of 6-G and other gingerols, as well as reduction of 6-S [7,11]. The extract obtained with the US and AM methods presented significantly lower antioxidant activity ($p < 0.05$) than the M, R1, R2, and R3 methods, due to shorter exposure time (40 min) and heat stress [14]. Moreover, according to Nguyen et al. [29], cavitation extraction is not strong enough to break the cell walls and release substances with antioxidant capacity.

On the other hand, the influence of solvent polarity on the extraction process was observed. Where the antioxidant capacity by FRAP of GFD extracts worked with different concentrations of methanol decreased (from 0.16 to 0.12 mmol $Fe^{2+}$/g dm), when the relative polarity of the extraction solvent was increased, as shown in Table 4. This is due to the decrease in the solubility of phenols, including 6-G and 6-S, as determined in other studies [5,24].

When comparing all the results of the extraction methods at different times, temperatures, and extraction mechanisms, a good correlation was observed between FRAP and 6-S (0.659) due to the fact that the structure of 6-S presents a double bond in the alkyl tail that can donate an electron to the ferric tripyridyl triazine complex, a compound present in the FRAP reagent [19], giving a better response of antioxidant capacity. Thus, method R presented a higher 6-S content (0.242–0.263 mg/g dm) and a higher antioxidant capacity by FRAP (0.179–0.192 mmol $Fe^{2+}$/g dm). On the other hand, the $IC_{50}$ in DPPH and 6-G presented a good negative correlation (−0.707), because the β-hydroxy keto group of the alkyl tail of 6-G can donate hydrogen to the DPPH free radical [20] and enhance the antioxidant capacity response. As observed in methods R4 and R5, with the lowest 6-G content (4.072–4.126 mg/g dm), they exhibited lower antioxidant capacity by $IC_{50}$ in DPPH (0.696–0.722 mg dm/mL).

Using the response surface curve, the 6-G and 6-S content and antioxidant capacity were related to extraction time and temperature. It was observed that at moderate temperatures and prolonged times, a higher 6-G content was obtained (Fig. 1a); conversaly, at high temperatures, regardless of the extraction time, a higher 6-S content was obtained (Fig. 1b). In addition, the antioxidant capacity by $IC_{50}$ in DPPH is related to lower 6-G content at prolonged times and elevated temperatures (Fig. 1c), and by FRAP is not related to time, but is related to higher extraction temperature (Fig. 1d), as reported for 6-S.

### 3.4. FTIR spectroscopy analysis of plant material with and without extraction

The FTIR analysis showed a reduction in the transmittance of the main functional groups characteristic of phenolics compounds and lignocellulosic material of ginger when it was subjected to the different extraction methods US, AM, M, and R1; being the spectrum belonging to method M the one that presented the lowest transmittance values suggesting a greater transformation of the surface of the plant material in relation to the spectrum of the GFD sample before the extraction process; this result is directly related to the higher antioxidant capacity present in the extract by method M, as shown in Fig. 2(a–e). The O–H bond at wavenumber 3250 $cm^{-1}$ corresponds to cellulose, hemicellulose, lignin, and phenolic compounds. The C–H bond of the $–CH_2$ and $–CH_3$ groups at wavenumbers 2890 and 2920 $cm^{-1}$, respectively, correspond to cellulose, hemicellulose, alkyl chains of phenols, and flavonoids. The C═C bond vibrations at wavenumber 1640 $cm^{-1}$ correspond to hemicellulose, lignin, and 6-S. The C–H bond at wavenumbers 1420 and 1240 $cm^{-1}$ corresponds to amines from hemicellulose and lignin. The C–O bonds at wavenumber 1000 $cm^{-1}$ are from the polysaccharides in cellulose. The C–*O*–C and C–*O*–H bonds at wave numbers 866 and 922 $cm^{-1}$, respectively, are from starch. These bonds are reduced as part of the cell wall transformation in ginger extraction processes, as reported by other authors [13].

### 3.5. Scanning electron microscopy SEM analysis

The GFD sample before the extraction process and after extraction by US, AM, M, and R1 methods were analyzed by SEM. The fresh sample analyzed by a light optical microscope from ginger rhizome showed the presence of oleoresin and amyloplasts containing

**Table 3**
Comparison of extraction methods on the antioxidant capacity of fresh air-dried ginger (GFD). Data represent mean ± standard deviation, n = 3.

| Extraction method | Time | Temperature °C | DPPH ($IC_{50}$ mg dry matter/mL) | FRAP (mmol $Fe^{2+}$/g dry matter) |
|---|---|---|---|---|
| US | 40 min | 40 | 0.668 ± 0.016 $^{cd}$ | 0.172 ± 0.003 $^{a}$ |
| AM | 40 min | 25 | 0.618 ± 0.005 $^{bc}$ | 0.180 ± 0.004 $^{ab}$ |
| M | 24 h | 25 | 0.581 ± 0.014 $^{ab}$ | 0.191 ± 0.003 $^{bc}$ |
| R1 | 1 h | 85 | 0.531 ± 0.019 $^{a}$ | 0.192 ± 0.004 $^{bc}$ |
| R2 | 2 h | 85 | 0.570 ± 0.062 $^{ab}$ | 0.188 ± 0.013 $^{bc}$ |
| R3 | 5 h | 85 | 0.561 ± 0.029 $^{ab}$ | 0.192 ± 0.003 $^{c}$ |
| R4 | 8 h | 85 | 0.696 ± 0.038 $^{d}$ | 0.187 ± 0.005 $^{bc}$ |
| R5 | 12 h | 85 | 0.722 ± 0.051 $^{d}$ | 0.179 ± 0.017 $^{ab}$ |

Different letters in the same column mean statistically different ($p < 0.05$) by Duncan's multiple range test.

**Table 4**
Influence of polarity on the antioxidant capacity of fresh air-dried ginger (GFD). Data represent mean ± standard deviation, n = 3.

| Solvent methanol in water | Relative polarity [30] | FRAP mmol $Fe^{2+}$/g |
|---|---|---|
| 100% | 0.76 | 0.168 ± 0.006 [a] |
| 80% | 0.81 | 0.153 ± 0.004 [b] |
| 70% | 0.83 | 0.143 ± 0.001[c] |
| 0% | 1.00 | 0.115 ± 0.003 [d] |

Different letters in the same column mean statistically different ($p < 0.05$) by Duncan's multiple range test.

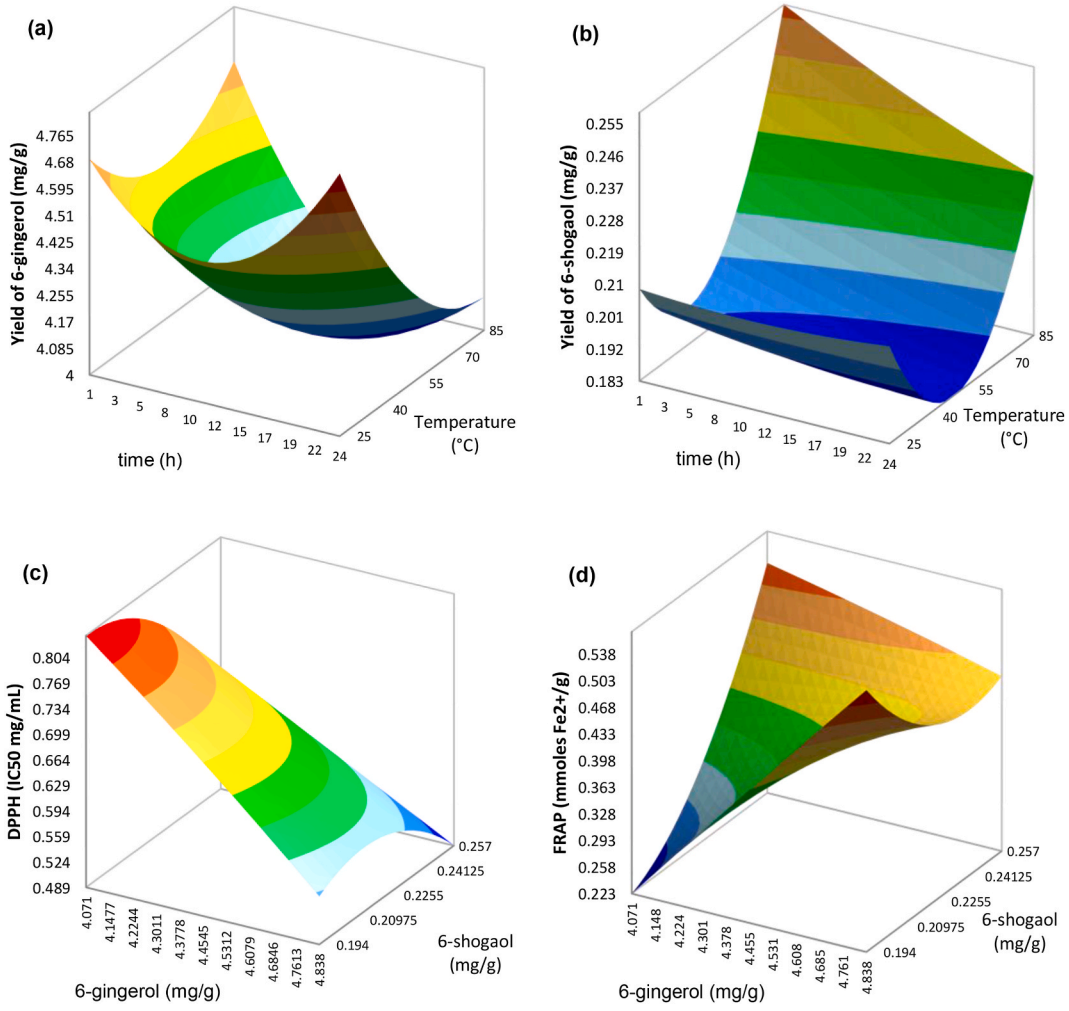


**Fig. 1.** Surface response (3D) of (a) 6-gingerol, (b) 6-shogaol content in relation to extraction time and temperature, and antioxidant capacity by (c) $IC_{50}$ in DPPH and (d) FRAP in relation to 6-gingerol and 6-shogaol content.

starch grains in the parenchyma cells. All extracted samples showed ruptured cell walls compared to the fresh sample, as shown in Fig. 3a-f.

The GFD samples subjected to the AM, M, and R1 methods showed more surface transformation of starch granules in the form of slight folds and ruptures compared to the US method and the fresh sample without extraction. This result is related to a higher antioxidant capacity of M and R1 compared to the US method, as long exposure time and moderate thermal stress favor the extraction of components with an antioxidant capacity [14]. Probably the cavitation force and the short extraction time were not enough to break the cell wall by the US method [14,28], being its result like the of GFD samples before the extraction process.

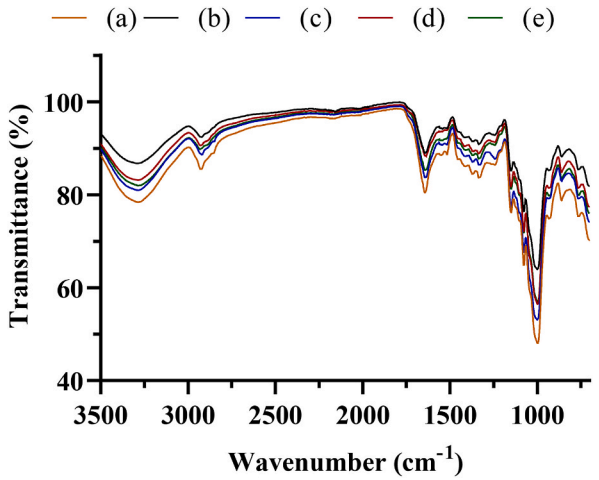


**Fig. 2.** Sample spectra of fresh air-dried ginger without extraction (a), with extraction by ultrasound method (b), magnetic stirring (c), maceration (d), and reflu×1 h (e).

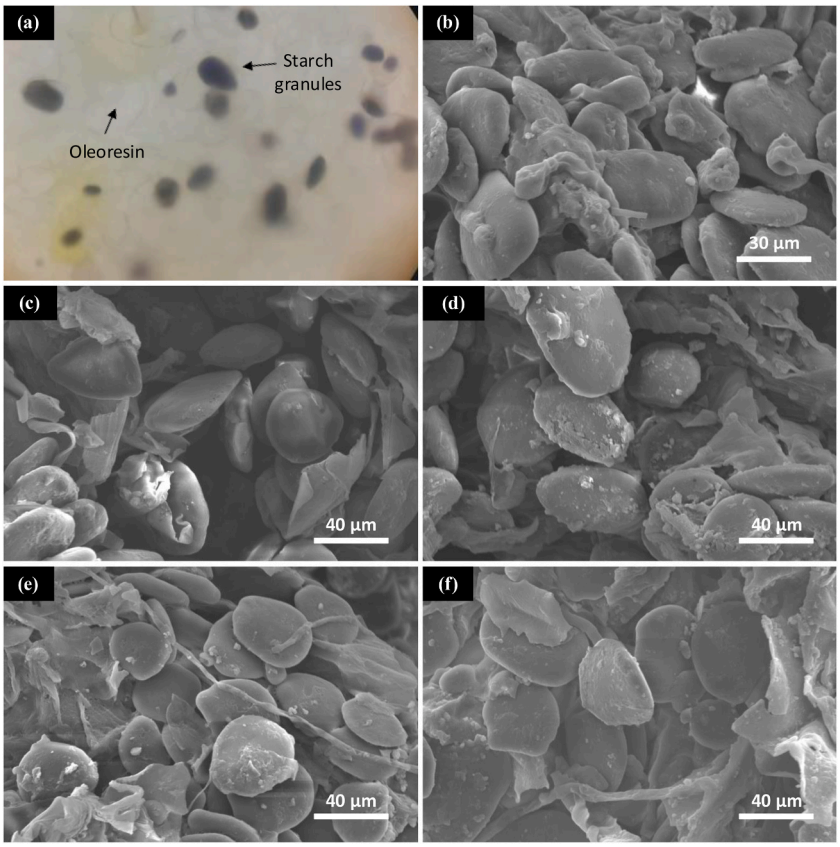


**Fig. 3.** Photo of fresh ginger (a), and SEM images: fresh air-dried ginger without extraction (b), with extraction by ultrasound method (c), magnetic stirring (d), maceration (e), and reflu×1 h (f).

## 4. Conclusions

Extracts of the GFD sample using solvents of medium relative polarity (0.76) such as methanol and by maceration (M) and reflux (R at 1 h) extraction methods presented higher antioxidant capacity by FRAP and $IC_{50}$ in DPPH compared to the magnetic stirring (AM), ultrasound (US) and reflux extraction methods at longer periods (12 h). Because, at longer exposure time and moderate heat stress in

the extracts, there will be a greater change in the structure and surface morphology in the starch granules, causing a higher extraction of bioactive compounds such as TFC, 6-G, and 6-S.

## Author contribution statement

Paola Aurelia Jorge Montalvo: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper. Carlos Vílchez-Perales: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data. Lizardo Visitación-Figueroa: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

## Data availability statement

Data will be made available on request.

## Additional information

No additional information is available for this paper.

## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References

[1] A.V. Vipin, K. Raksha, K. Nawneet, A. Anu, G. Venkateswaran, Protective effects of phenolics rich extract of ginger against Aflatoxin B1-induced oxidative stress and hepatotoxicity, Biomed. Pharmacother. 91 (2017) 415–424.
[2] H. Yeh, C. Chuang, H. Chen, C. Wan, T. Chen, L. Lin, Bioactive components analysis of two various gingers (*Zingiber officinale* Roscoe) and antioxidant effect of ginger extracts, LWT–Food Sci. Technol. 55 (2014) 329–334.
[3] Y. Li, Y. Hong, Y. Han, Y. Wang, L. Xia, Chemical characterization and antioxidant activities comparison in fresh, dried, stir-frying and carbonized ginger, J. Chromatogr., B: Anal. Technol. Biomed. Life Sci. 1011 (2016) 223–232.
[4] W. Si, Y.P. Chen, J. Zhang, Z.Y. Chen, H.Y. Chung, Antioxidant activities of ginger extract and its constituents toward lipids, Food Chem. 239 (2018) 1117–1125.
[5] K.D. Prasanna, P. Gunathilake, H.P. Vasantha, Inhibition of human low-density lipoprotein oxidation in vitro by ginger extracts, J. Med. Food 17 (2014) 424–431.
[6] S. Ok, W.S. Jeong, Optimization of extraction conditions for the 6-shogaol-rich extract from ginger (*Zingiber officinale* Roscoe), Prev. Nutr. Food Sci. 17 (2012) 166–171.
[7] J. Jhonson, J. Mani, S. White, P. Brown, M. Naiker, Quantitative profiling of gingerol and its derivatives in Australian ginger, J. Food Compos. Anal. 104 (2021) 1–8.
[8] M.J. Ko, H.H. Nam, M.S. Chung, Conversion of 6-gingerol to 6-shogaol in ginger (*Zingiber officinale*) pulp and peel during subcritical water extraction, Food Chem. 270 (2019) 149–155.
[9] A. Ghasemzadeh, H.Z.E. Jaafar, A. Rahmat, Optimization protocol for the extraction of 6-gingerol and 6-shogaol from *Zingiber officinale* var. rubrum Theilade and improving antioxidant and anticancer activity using response surface methodology, BMC Compl. Alternative Med. 15 (2015) 1–10.
[10] A. Jelled, A. Fernandes, L. Barros, H. Chahdoura, L. Achour, I.C.F.R. Ferreira, H. Ben Cheikh, Chemical and antioxidant parameters of dried forms of ginger rhizomes, Ind. Crop. Prod. 77 (2015) 30–35.
[11] U. Griškevičienė, M. Marksa, A. Ževžikovienė, D. Kazlauskienė, R. Vainorienė, A. Ževžikovas, L. Ivanauskas, *Cirsium vulgare* leaves: isolation and identification of phenolic compounds, Chemija 32 (2021) 92–99.
[12] K. Grabowska, K. Amanowicz, P. Paśko, I. Podolak, A. Galanty, Optimization of the extraction procedure for the phenolic-rich *Glechoma hederacea* L. Herb and evaluation of its cytotoxic and antioxidant potential, Plants 11 (2022) 1–14.
[13] S. Eyupoglu, Characterization of new cellulosic fibers obtained from *Zingiber officinale*, J. Nat. Fibers (2020) 1–10.
[14] W. Liu, C. Zhou, J. Zhao, D. Chen, Q. Li, Optimized microwave-assisted extraction oj 6-gingerol from *Zingiber officinale* Roscoe and evaluation of antioxidant activity in vitro, Acta Sci. Pol.,Technol. Aliment. 13 (2014) 155–168.
[15] P.A. Jorge, L.F. Ortiz, J.L. Calle, L.A. Téllez, M.F. Césare, L. Visitación, Transformación del nitrógeno durante el compostaje de bosta de caballo, Prod. + Limpia. 13 (2018) 77–88.
[16] C.O. Eleazu, K.C. Eleazu, Physico-chemical properties and antioxidant potentials of 6 new varieties of ginger (Zingiber officinale), Am. J. Food Technol. 7 (2012) 214–221.
[17] A. Sangwan, A. Kawatra, S. Sehgal, Nutritional composition of ginger powder prepared using various drying methods, J. Food Sci. Technol. 51 (2012) 2260–2262.
[18] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, Methods Enzymol. 299 (1999) 152–178.
[19] I.F. Benzie, J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay, Anal. Biochem. 239 (1996) 70–76.
[20] L.L. Mensor, F.S. Menezes, G.G. Leitao, A.S. Reis, T.C. Dos Santos, C.S. Coube, S.G. Leitao, Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method, Phyther. Res. 15 (2001) 127–130.
[21] R.A. Naveda, P.A. Jorge, L. Flores, L. Visitación, Remoción de lignina en el pretratamiento de cascarilla de arroz por explosión con vapor, Rev. Soc. Química Del Perú. 85 (2019) 352–361.
[22] O.B. Ajayi, S.F. Akomolafe, F.T. Akinyemi, Food value of two varieties of ginger (*Zingiber officinale*) commonly consumed in Nigeria, ISRN Nutr 2013 (2013) 1–5.
[23] C.E.C. Ugwoke, U. Nzekwe, Phytochemistry and proximate composition of ginger (Zingiber officinale), J. Pharmaceut. Allied Sci. 7 (2010) 1182–1187.

[24] A. Shukla, V.V. Goud, C. Das, Antioxidant potential and nutritional compositions of selected ginger varieties found in Northeast India, Ind. Crop. Prod. 128 (2019) 167–176.
[25] G. Chen, B. Yuan, H. Wang, G. Qi, S. Cheng, Characterization and antioxidant activity of polysaccharides obtained from ginger pomace using two different extraction processes, Int. J. Biol. Macromol. 139 (2019) 1–9.
[26] P. Jorge-Montalvo, C. Vílchez-Perales, L. Visitación-Figueroa, Propiedades farmacológicas del jengibre (*Zingiber officinale*) para la prevención y el tratamiento de COVID-19, Agroindustr. Sci. 2 (2020) 329–338.
[27] N. Utama-ang, S. Sida, P. Wanachantararak, A. Kawee-ai, Development of edible Thai rice film fortified with ginger extract using microwave-assisted extraction for oral antimicrobial properties, Sci. Rep. 11 (2021) 1–10.
[28] T. Žitek, N. Kučuk, V. Postružnik, M. Leitgeb, Ž. Knez, M. Primožič, M.K. Marevci, Synergistic effect of supercritical and ultrasound-assisted ginger (*Zingiber officinale* Roscoe) extracts, Plants 11 (2022) 1–14.
[29] S.T. Nguyen, P.H. Vo, T.D. Nguyen, N.M. Do, B.H. Le, D.T. Dinh, K.D. Truong, P. Van Pham, Ethanol extract of ginger *Zingiber officinale* Roscoe by Soxhlet method induces apoptosis in human hepatocellular carcinoma cell line, Biomed. Res. Ther. 6 (2019) 3433–3442.
[30] C. Reichardt, T. Welton, Solvents and solvent effects in organic chemistry, 4th Editio, Germany, 2010.