Production of a gingerol-rich extract from ginger rhizome powder using a green extraction protocol

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Summary
Ginger has a long history of use as a pungent spice for food and medicinal applications. Drying and grinding of fresh ginger lead to a decrease in its major pungent components, gingerols, and to an increase in shogaols (dehydration isomers). The present study was designed to prepare a gingerols-rich extract from ginger powder using a green extraction protocol. α-Amylase was used to digest starch before extraction of powdered ginger and several factors influencing the process, in particular enzyme concentration, incubation time, extraction temperature and solvent effect were investigated. Extraction of the powder with water gave the extract poor in both 6-gingerol and 6-shogaol, extraction with 60% glycerol led to a high yield of 6-gingerol and a low yield of 6-shogaol, while extraction with 70% ethanol yielded the extract rich in 6-shogaol but relatively poor in 6-gingerol. Enzymatic digestion of ginger powder with α-amylase for 3 days before extraction with 60% glycerol gave an extract rich in 6-gingerol (31.01 $g \cdot kg^{-1}$), which was approximately 45-fold higher than the aqueous extract (0.66 $g \cdot kg^{-1}$). Accordingly, enzymatic digestion of starch before extraction of ginger powder with glycerol is an efficient protocol for producing gingerols-rich extract, as determined by high-performance liquid chromatography.

Keywords
ginger; *Zingiber officinale*; 6-gingerol; 6-shogaol; α-amylase; glycerol

Ginger, the rhizome of *Zingiber officinale* Roscoe, contains a number of biologically active pungent compounds, the most notable of which are 6-gingerol, 6-shogaol and zingerone [1]. Gingerols (particularly 6-gingerol) are the most abundant pungent compounds in fresh rhizomes and were reported to exert a wide range of biological activities [2–5]. Various methods have been used to extract the bioactive compounds from ginger, such as infusion, decoction, reflux, sonication, hydrodistillation, steam distillation, leaching, pressing, supercritical carbon dioxide or high-pressure Soxhlet extraction (HPSE) [6–9]. Guo et al. [10] developed an ionic liquid-based microwave-assisted extraction method to extract 6-, 8-, 10-gingerols and 6-, 8-, and 10-shogaols from ginger. The drying and extraction processes can have an impact on the composition and functionality of the ginger extracts. Alternative methods for obtaining ginger extracts with high gingerol and shogaol contents were reported [7, 11]. The composition and bioactivity of the ginger extract are affected by drying, extraction and complementary processes, e. g. enzymatic-assisted extraction, acidic and carbonic maceration.

Lower temperature extraction methods, such as cold ultrasound-assisted extraction, produce extracts with higher levels of phenolics, gingerols and a higher antioxidant activity. On the other hand, acidic solvents or "hot" processes, such as microwave-drying, pressurized liquid extraction or microwave-assisted extraction, can tend to favour higher shogaol concentrations, which have greater antitumour, anti-inflammatory and antimicrobial activities than gingerols [12].

Several organic solvents, namely, ethanol,

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acetone, hexane and sometimes chloroform were used to extract ginger at laboratory and industrial levels. However, scarce information is available concerning the use of glycerol for the same application, though it is commonly used in pharmacy and food technology [13]. It is characterized by being similar to ethanol but not flammable, water-miscible, biodegradable, non-toxic, having low volatility and low glycemic index [14]. These properties, in addition to its low price, qualify the use of glycerol for extraction of bioactive constituents with health-promoting activities.

The application of glycerol–water mixture as a solvent system for extraction of bioactive components from plants have been expanded into various industrial technologies and became common for the extraction of polyphenolics [7, 11, 12, 15]. Since glycerol can be produced from waste products or from renewable sources, this substance has a high potential to serve as an alternative green solvent for organic reactions [16].

The starch content of ginger powder is almost 500 g·kg^{-1} [17] and its presence in the powder may hinder efficient extraction of the main bioactive compounds. Accordingly, it is important to adopt a chemical or biological digestion pre-treatment for the removal of starch to facilitate the extraction of the target bioactive compounds. Also, the processes followed should enable their application in the food or pharmaceutical industries. Biological approaches for starch digestion have several advantages over non-biological ones, such as being environmentally friendly by no use of organic or corrosive solvents and reagents.

The aim of the current study was to develop an optimal extraction protocol to produce a 6-gingerol-rich extract from ginger powder. This extraction protocol involved three major consecutive steps: 1) pre-treatment digestion of the ginger powder using α-amylase enzyme before extraction, 2) extraction of the digested powder with glycerol and 3) concentrating the extract using a styrene divenylbenzene column.

MATERIALS AND METHODS

Plant material and chemicals

A sample of powdered ginger (*Zingiber officinale* Roscoe) was supplied by Natural Wellness (Cheras, Kuala Lumpur, Malaysia) in 2020. The standards (6-gingerol and 6-shogaol) for high performance liquid chromatography (HPLC) analysis were obtained from Nawah Scientific (Mokatam, Cairo, Egypt). Diaion HP20 column (75 g, 60.0 cm × 3.5 cm, Mitsubishi Chemical Industries, Tokyo, Japan) was used for chromatographic separation. Porcine pancreatic α-amylase (EC 3.2.1.1) was obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Phosphate buffer solution (pH 6) was obtained from Maadi Medical Supplies (Cairo, Egypt). All other reagents used in this study were of analytical grade and the highest purity available.

Standard calibration curves

Standard calibration curves for quantitation of 6-gingerol and 6-shogaol were constructed using solutions containing five concentrations of the standard 6-gingerol (50–1000 μg·ml^{-1}) in methanol. For quantitation of 6-shogaol, a calibration curve was obtained from solutions of the standard at four concentrations (50–500 μg·ml^{-1}). Both calibration curves were obtained from the chromatographic peak areas at each concentration level of the solutions evaluated in triplicate and the line equations generated from the averages of these values are given in Eq. 1 for gingerol and in Eq. 2 for shogaol.

$$y = 21.32x + 778.5 \quad R^2 = 0.9974 \tag{1}$$

$$y = 43.94x + 2291.3 \quad R^2 = 0.9911 \tag{2}$$

Extraction procedures

Aqueous and ethanol extracts

Samples (10 g each) of ginger powder were separately extracted with 70% ethanol (1 : 10, fraction marked as E70) using an ultrasonic bath Transsonic TS 540 (Elma, Singen, Germany) for 30 min, and 100 ml of hot (100 °C) distilled water for 10 min, followed by sonication for 30 min at 37 °C (1 : 10, fraction marked as W1). Both extracts were then separately filtered through Whatman No. 1 filter paper (Whatman, Maidstone, United Kingdom), evaporated, and analysed by HPLC to determine 6-gingerol and 6-shogaol contents. Fraction W1 was then chromatographed on a Diaion HP20 column sequentially eluted with distilled water (500 ml) and ethanol (200 ml). The ethanolic fraction (W2) from the Diaion HP20 column was then evaporated using a rotary evaporator R210 (Buchi, Flawil, Switzerland) under reduced pressure at 50 °C. The contents of gingerol and shogaol were determined by HPLC. The flow chart of the process is presented in Fig. 1.

Glycerol extracts

Samples (10 g each) of ginger powder were separately extracted with glycerol (40% v/v or 60% v/v) at a powder-to-solvent ratio of (1 : 10) using sonication for 30 min. The glycerol extracts G1 (extracted with 40% glycerol, v/v) and

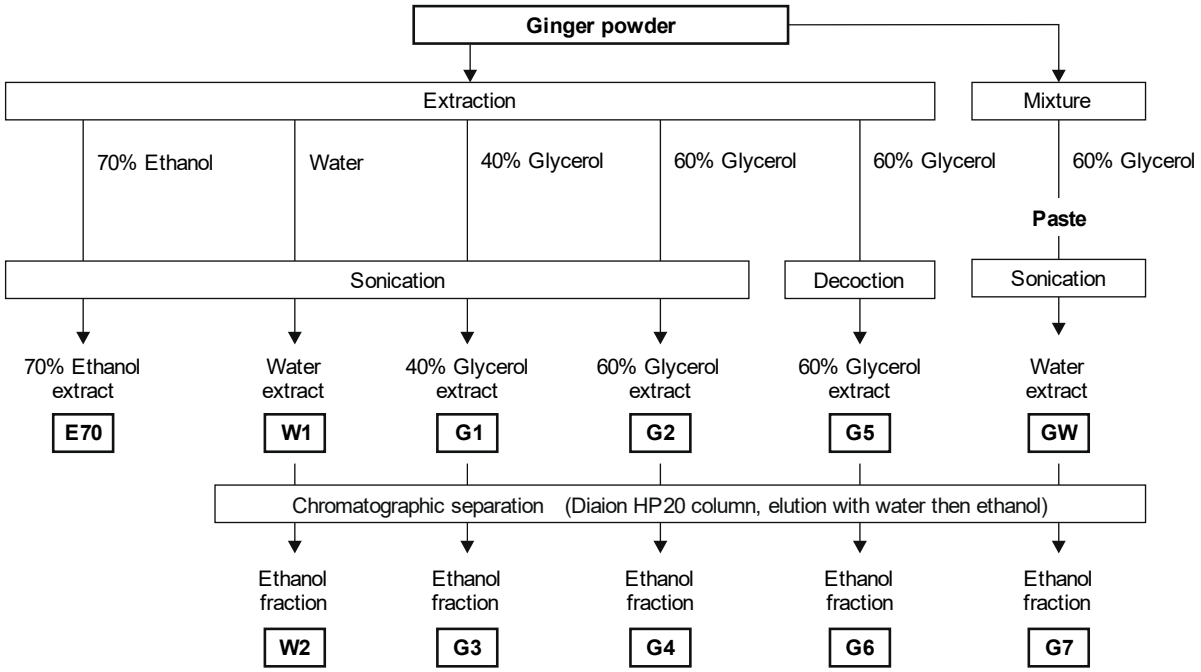

Fig. 1. Process flow chart for extraction of 6-gingerol and 6-shogaol from ginger powder using various solvents and extraction methods.

G2 (extracted with 60% glycerol, v/v) were then separately filtered through Whatman No. 1 filter paper using a Buchner funnel, and fractionated on a Diaion HP20 column with distilled water (750 ml) to eliminate the starch and glycerol content, then followed by ethanol (200 ml). The ethanolic fractions G3 (extracted with 40% glycerol, v/v) and G4 (extracted with 60% glycerol, v/v) from the Diaion HP20 column were then evaporated using a rotary evaporator under reduced pressure at 50 °C, and their contents of gingerol and shogaol were determined by HPLC.

Then, based on gingerol and shogaol content, the best glycerol concentration was further used in further extraction methods:

- Decoction method: a sample (10 g) of ginger powder was extracted with 60% glycerol, v/v (1 : 10, fraction G5) over 3 h in a water bath at 75 °C.
- Paste method: a sample (10 g) of ginger powder was mixed with 60% glycerol, v/v (1 : 2) to make a paste, which was then dried in a vacuum oven at 40 °C for 24 h and extracted with water (1 : 10, fraction GW) with support of ultrasound for 30 min.

All extracts from each treatment were subjected to the same procedure as described above for the glycerol extract obtained via sonication to obtained ethanolic Diaion fractions G6 and G7 for decoction and paste methods, respectively. A flow chart of the process is presented in Fig. 1.

Enzymatic hydrolysis

Thirty grams of powdered ginger were separately treated at 1 : 10 with α-amylase at 0.5 $U \cdot ml^{-1}$ or 5 $U \cdot ml^{-1}$ in a phosphate buffer, pH 6.0 with 0.20 $g \cdot l^{-1}$ NaN_3). The mixture was incubated in a shaking incubator SI 500 (Stuart, Stone, United Kingdom) at 37 °C and 20 Hz for 3 days. The filtrates for each treatment were then, separately, subjected to Diaion HP20 column sequentially eluted with water (500 ml) and ethanol (200 ml) to obtain ethanolic Diaion fractions FB, FE1 and FE2 for the buffer and enzyme filtrates at low and high concentrations, respectively. The marc for each treatment was dried using a vacuum oven at 40 °C for 24 h and then extracted with 60% glycerol (v/v) at 75 °C during 3 h.

The extracts for each treatment were then chromatographed on a Diaion HP20 column sequentially eluted with water (750 ml) and ethanol (200 ml). The ethanolic fractions MB (buffer), ME1 (0.5 U ml^{-1} enzyme) and ME2 (5 U ml^{-1} enzyme) from the chromatographic column were then evaporated using a rotary evaporator under reduced pressure at 50 °C and their contents of gingerol and shogaol were determined by HPLC. The flow chart of the process is presented in Fig. 2.

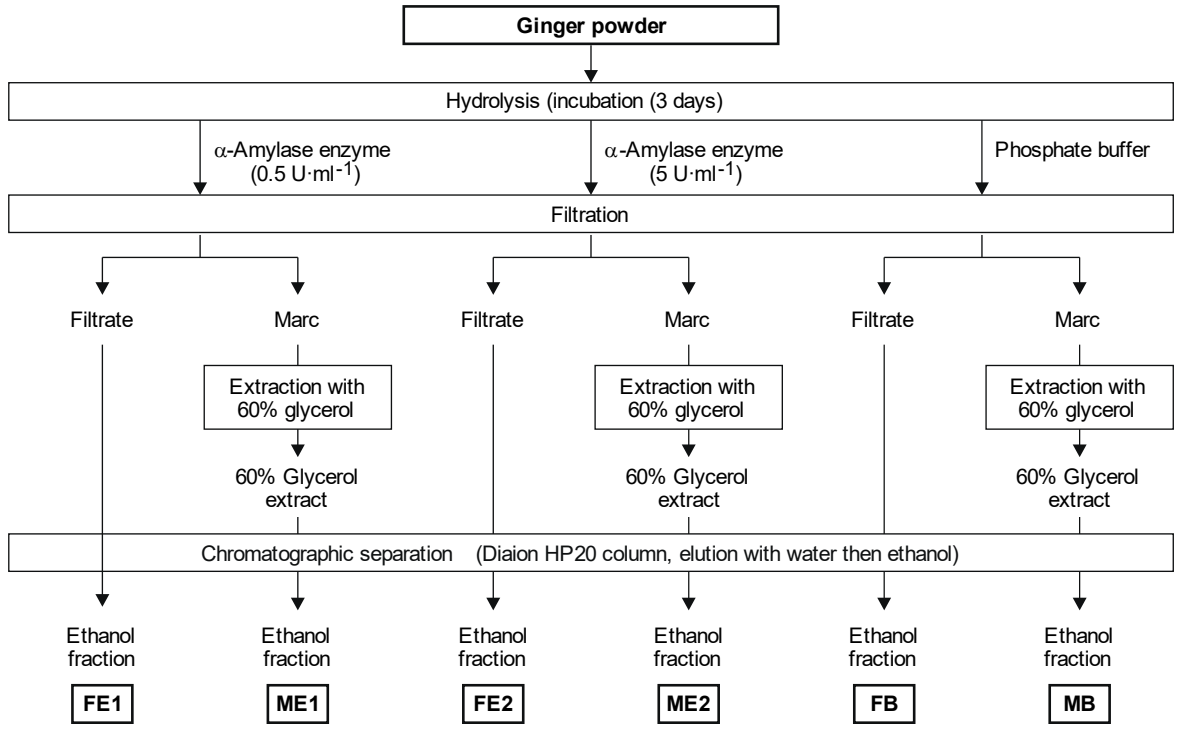

Fig. 2. Process flow chart for extraction of 6-gingerol and 6-shogaol from ginger powder using enzymatic digestion with α-amylase.

Determination of 6-gingerol and 6-shogaol

Agilent 1200 Infinity HPLC instrument (Agilent Technologies, Santa Clara, California, USA) equipped with an automatic injector and diode array detector (DAD) was used for the HPLC determination of 6-gingerol and 6-shogaol contents (Fig. 3) using Kromasil RP-18 column (4.6 mm × 250 mm, particle size 5 mm, pore size 10 nm; Nouryon, Göteborg, Sweeden). Gradient chromatographic separation was carried out using a mobile phase composed of A (acetonitrile) and B (1 $g \cdot l^{-1}$ triflouroacetic acid in water). The gradient elution program was: 35 % A (0–2 min), 35–60 % A (2–10 min) and 60–100 % A (10–12 min). The flow rate was 1.0 $ml \cdot min^{-1}$, the peaks were monitored at 230 nm and 278 nm. 6-Gingerol and 6-shogaol were used as external standards.

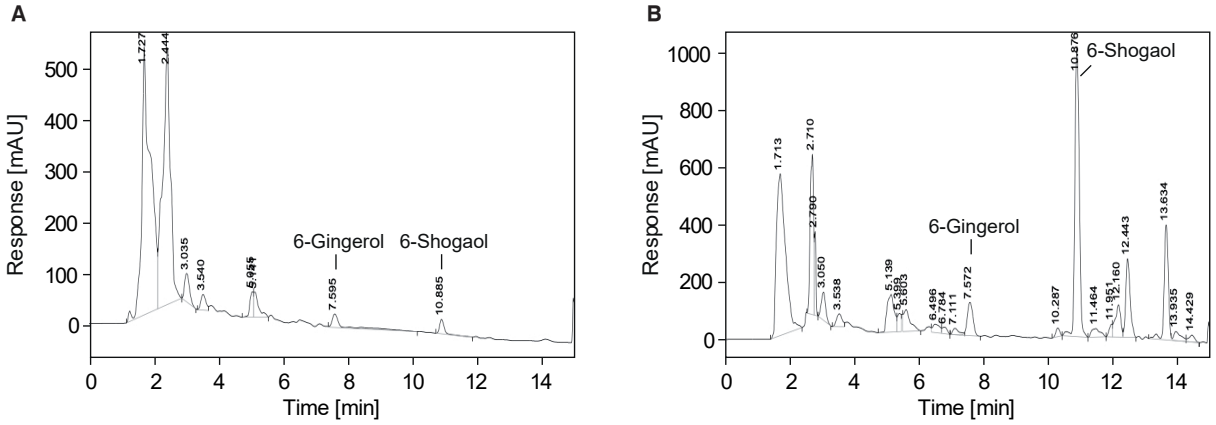

Fig. 3. HPLC chromatograms of ginger extracted using various solvents.

A – aqueous extraction, B – extraction with 70% ethanol (v/v).

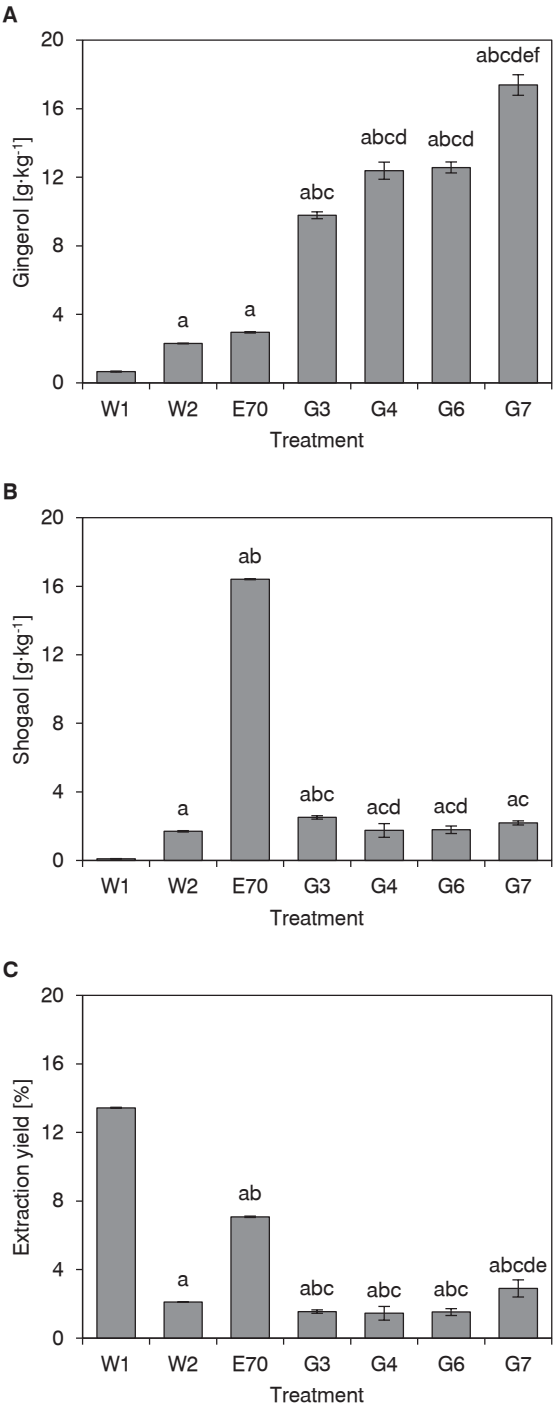

Fig. 4. Gingerol content, shogaol content and extraction yield after extraction of ginger powder using various solvents.

A – gingerol content, B – shogaol content, C – extraction yield.
Treatments designation is explained in Fig. 1.
Letters above bars indicate statistical significance at $p < 0.05$ (a – significantly different from W1, b – significantly different from W2, c – significantly different from E70, d – significantly different from G3, e – significantly different from G4, f – significantly different from G7).

Statistical analysis

All data were represented as mean ± standard deviation. The significance of the difference between means was analysed using one-way ANOVA followed by Tukey's test by GraphPad Prism 9 (Dotmatics, San Diego, California, USA).

RESULTS AND DISCUSSION

The selection of the best extraction solvent is one of the most important factors affecting extraction efficiency. HPLC analysis was performed to determine the contents of 6-gingerol and 6-shogaol in extracts obtained with various solvents, using standard calibration curves for both compounds. The results were compared to those obtained in the same extraction conditions using water and ethanol as solvents.

In general, 6-gingerol and 6-shogaol are non-polar compounds, so their yields in the water extracts are low, as shown in Fig. 4. The yields of 6-gingerol and 6-shogaol contents in E70 extract revealed that dried ginger powder contained more 6-shogaol than 6-gingerol, which is considered normal in dried ginger powder due to dehydration of gingerol to shogaol during drying and subsequent extraction processes.

Fig. 1 illustrates the extraction procedure before the reduction of starch content. Comparing the results from all tested extraction methods, extraction using 60% glycerol was the most efficient in terms of 6-gingerol content. However, extraction with water gave W1 with low levels of gingerol and shogaol, respectively (0.66 $g \cdot kg^{-1}$ and 0.10 $g \cdot kg^{-1}$).

Concentrating W1 by using a Diaion HP20 column eluted with water followed by ethanol increased this content to 2.3 $g \cdot kg^{-1}$ and 1.7 $g \cdot kg^{-1}$ (dry extract basis, W2) of gingerol and shogaol, respectively, while the yield of the extract was decreased from 13.40 $g \cdot kg^{-1}$ to only 2.10 $g \cdot kg^{-1}$ (plant powder basis). The extraction with 70% ethanol showed an increase in both compounds to 2.95 $g \cdot kg^{-1}$ and 16.41 $g \cdot kg^{-1}$ (dry extract basis) of 6-gingerol and 6-shogaol, respectively, with an extractive yield of 71.00 $g \cdot kg^{-1}$ (plant powder basis). Extraction with 40% glycerol and sonication for 30 min resulted in a good yield of gingerol with a content of 9.78 $g \cdot kg^{-1}$ (dry extract basis, G3) compared to a lower yield of shogaol with a content of 2.51 $g \cdot kg^{-1}$ (dry extract basis). Increasing the glycerol concentration to 60 % resulted in an increase in gingerol content to 12.38 $g \cdot kg^{-1}$ (dry extract basis, G4), while shogaol showed similar results to the previous concentration.

Based on these results, the optimal extraction using 60% glycerol with different extraction techniques such as heating to 70 °C or making a paste and incubation overnight at 40 °C was carried out. Using paste with glycerol in extraction increased the gingerol content to 17.38 g·kg^{-1} (dry extract basis). Regarding the percentage extract yield from the plant powder, G3, G4, G6 and G7 showed similar values of 15 g·kg^{-1}, 14 g·kg^{-1}, 29 g·kg^{-1} and 15 g·kg^{-1}, respectively.

6-Shogaol is significantly more lipophilic than 6-gingerol due to the loss of a hydroxyl group from gingerol during dehydration. Lipophilicity differences between 6-gingerol and 6-shogaol would result in significant differences in solubility in glycerol, with H-bonding interactions affecting extractability of the compounds by gylcerol.

The α-amylase enzyme was used in two concentrations; 0.5 U·ml^{-1} and 5 U·ml^{-1}, and the content of both gingerol and shogaol were determined both in the filtrate and in the digested powder. The filtrate of enzymatically digested ginger powder (FE1) yielded a higher content of 6-gingerol (9.44 g·kg^{-1}, extract basis) at the lower enzyme concentration (0.5 U·ml^{-1}) compared to that obtained when higher enzyme concentration was used, or in the blank filtrate without enzyme (gingerol contents of 4.55 g·kg^{-1} and 2.58 g·kg^{-1}, dry extract basis, respectively).

Ginger powder after enzymatic digestion with the enzyme at a concentration of 0.5 U·ml^{-1} was extracted with 60% glycerol (as a paste) to give extract with the highest gingerol content (ME1, 21.57 g·kg^{-1} extract) in all treatments, compared to its content (17.89 g·kg^{-1} dry extract) when 5 U·ml^{-1} enzyme concentration was used. On the other hand, higher gingerol content of 31.01 g·kg^{-1} (extract basis) was obtained after processing both filtrates and the digested marc using Diaion HP20 eluted with ethanol.

The use of enzyme-assisted extraction in the preparation of phytoconstituents-rich extracts from natural products is a quite common technique. Nagendra Chari et al. [18] reported that the digestion of ginger powder using α-amylase or viscozyme followed by acetone extraction resulted in a two-fold increase in gingerol yield (from 64 g·kg^{-1} to 12.2 g·kg^{-1}). Also, enzymatic digestion of ginger powder followed by three-phase partitioning comprising water, ammonium sulfate and butanol resulted in a marked increase in the yield of gingerol in the extract [19]. In the current study, using α-amylase enzyme was found to increase the extractability of gingerol in the digested marc, i. e. increase of gingerol content in both the filtrate of the digestion buffer and the powder/marc after

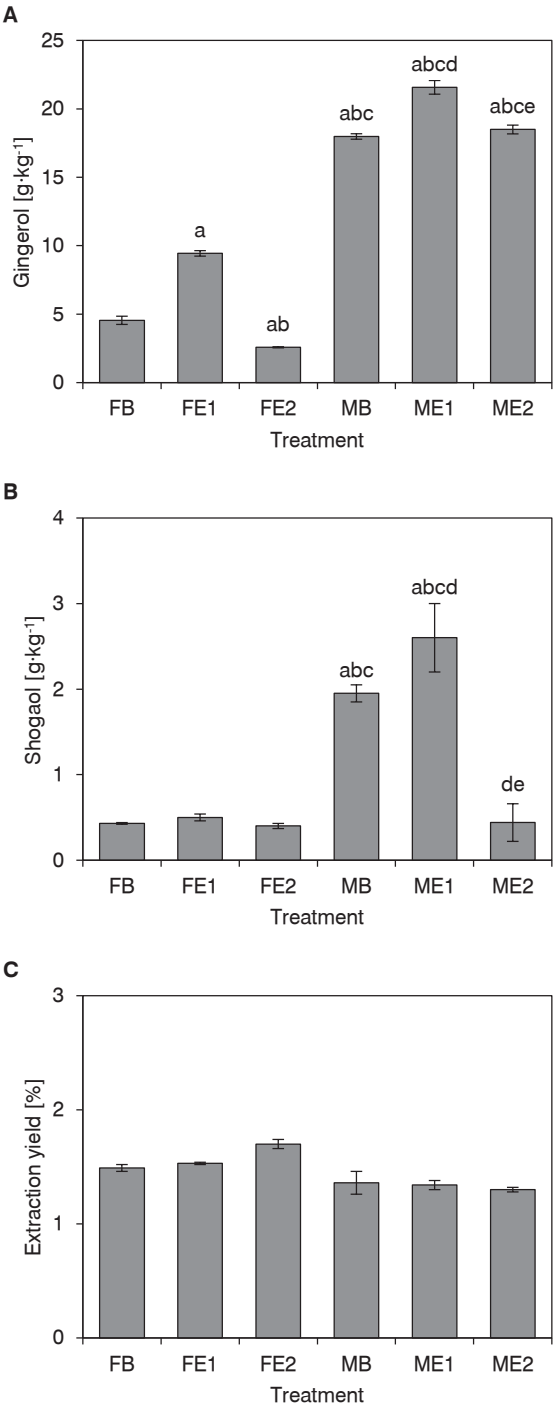

Fig. 5. Gingerol content, shogaol content and extraction yield after enzymatic hydrolysis of ginger powder.

A – gingerol content, B – shogaol content, C – extraction yield.
Treatments designation is explained in Fig. 2.
Letters above bars indicate statistical significance at $p < 0.05$ (a – significantly different from FB, b – significantly different from FE1, c – significantly different from FE2, d – significantly different from MB, e – significantly different from ME1, f – significantly different from ME2).

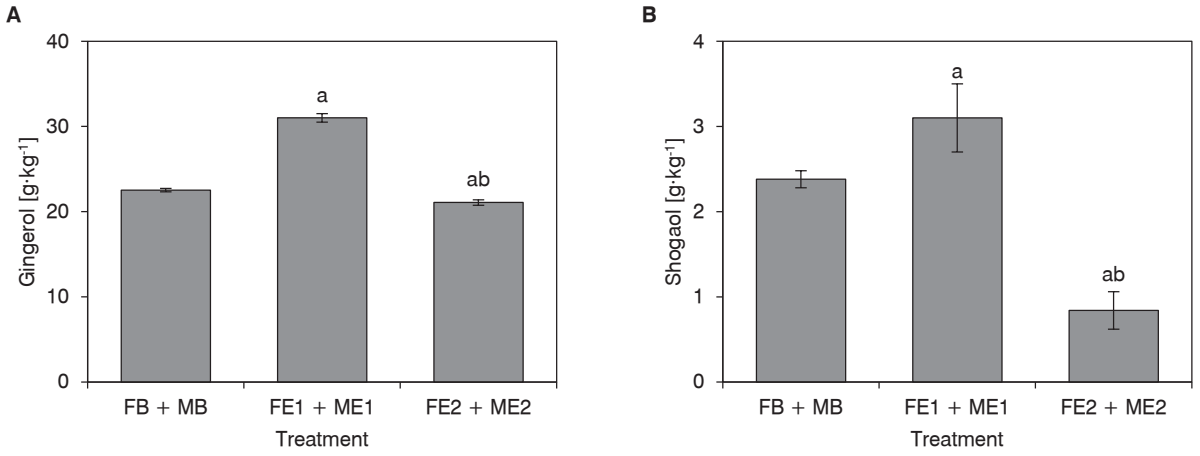

Fig. 6. Total gingerol and shogaol contents in powder extract and filtrate after enzymatic hydrolysis.

A – total gingerol content, B – total shogaol content.
Treatments designation is explained in Fig. 2.
Letters above bars indicate statistical significance at $p < 0.05$ (a – significantly different from FB + MB, b – significantly different from FE1 + ME1, c – significantly different from FE2 + ME2).

extraction with aqueous glycerol (Fig. 5, Fig. 6). Similar findings were previously reported about the effect of enzyme digestion on gingerol extractability. Moreover, hazardous organic solvents were not used for extraction in the current method, which is considered a green extraction method.

Several reports dealt with the optimization of the extraction of ginger powder using various solvents, extraction conditions and techniques [6–8, 10, 20, 21]. The use of hydrothermal and high-pressure extraction methods markedly improved gingerol and shogaol content. However, using enzyme-assisted extraction resulted in a fruity taste of the hydrolysed powder [20]. The effect of 70% ethanol (v/v), various temperatures and various times of extraction on shogaol and gingerol contents was studied by Cha et al. [22]. Gingerol extract of 35 g·kg^{-1} (dry extract basis) was obtained by extraction with 70% ethanol (v/v) for 70 min at 70 °C. The shogaol content was 2.44 g·kg^{-1} (dry extract basis) using 70% ethanol (v/v) for 51.90 min at 62.29 °C. In a different report by Ghasemzadeh et al. [23], the optimum extraction temperature was found to be 76.9 °C and the duration 3.4 h that yielded an alcoholic extract with a low content of gingerol and shogaol, (2.89 g·kg^{-1} and 1.85 g·kg^{-1}, dry extract basis, respectively). Liu et al. [24] found that the optimum conditions for ginger extraction were in the order of high-pressure extraction > high-temperature extraction > blender extraction > low-pressure extraction. In addition, 95% ethanol (v/v) was found to be the best solvent for ginger extraction. Using microwave-assisted extraction by 70% ethanol (v/v) for 10 min at 180 W an extract was obtained containing 2.8 g·kg^{-1} and 1.3 g·kg^{-1}, dry extract basis, of gingerol and shogaol, respectively [25]. Enzyme-assisted extraction of ginger powder with unadjusted pH followed by reflux with ethanol for 2 h at 83 °C revealed an extract with 65 g·kg^{-1} of total gingerol and shogaol [26]. Ginger extract with 0.68 g·kg^{-1} of gingerol and 0.39 g·kg^{-1} dry extract of shogaol was obtained by applying subcritical water extraction as an eco-friendly method [11]. Increasing the temperature and time in this method favoured the production of shogaol-rich extract due to the dehydration of gingerol by high temperature [11]. Compared to the previous data, the method used in the current study can be ranked as one of the best methods in terms of the yield of gingerol with the advantage of not using organic solvents in the extraction process, which makes it a more environmentally friendly method. Overall, in the current study, the achieved 6-gingerol content in the aqueous extract of ginger powder digested with α-amylase at a low concentration was 10-fold higher than that in the extract of the powder digested with the enzyme at a higher concentration. After enzyme digestion, the aqueous filtrate extract had a 2-fold increase in the 6-gingerol content compared to that of the unprocessed powder, i. e. treated with only the buffer solution (2.30 g·kg^{-1} against 1.70 g·kg^{-1} extract). This means that the starch content has a significant effect on the extraction process. These data supported the positive role of α-amylase. α-Amylases are ubiquitous natural products, distributed in bacteria, fungi, animals and plants. These enzymes are essen-

tial for the digestion of starch and glycogen, and have a broad application in food and feed area.

Utilization of enzymes is an eco-friendly process that produces a small amounts of by-products, requires less energy and allows the use of simple downstream operations. However, their cost-effective production is still a big challenge. Application of biotechnological approaches and the use of a low-cost plant-based biomass for their production appears to be the most effective approach to solve these problems.

CONCLUSIONS

The extraction method developed in this study provided an extract rich in gingerol from a powder rich in shogaol. The results presented demonstrate that reducing the starch content of the ginger powder using enzymatic digestion followed by extraction with 60% glycerol (v/v) is an efficient method for increasing the extractability of 6-gingerol from the ginger powder in terms of yield and content. Also, processing of the extract on a column of Diaion HP20 yielded an extract rich in gingerol. A different extraction efficiency was observed using 70% ethanol (v/v), when an extract rich in 6-shogaol was obtained.

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