Research Article

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Variation in bioactive phytochemicals and sensory attributes of osmosonic convective dried ginger from four African countries

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Abstract

BACKGROUND: The rhizome of ginger (*Zingiber officinale* Roscoe) is one of the most patronized spices worldwide and plays an important role in folklore medicine. In this study, we aimed to determine the quality of ginger samples from representative West African (Ghana, Nigeria) and East African (Uganda, Kenya) countries. By that, we also implicitly sought to determine the probable influence of location of cultivation (and the intrinsic growth conditions) on the quality of the samples. The ginger samples were pretreated by osmosonication prior to relative humidity convective drying and analyzed for differences in their metabolomes, total phenolic content (TPC) and total flavonoid content (TFC), antioxidant activities, sensory characteristics and volatile compounds composition (via electronic-nose determination).

RESULTS: The outcome of our study showed marked source-dependent differences in the metabolomes of the samples as captured by a metabolomics approach. Based on the findings of the metabolomics study, 6-gingerol content was quantified and found to be higher in the samples of West African origin. Also, the samples from the two West African countries contained higher levels of bioactive phytochemicals as evinced by the results of TPC, TFC, e-nose analysis, and antioxidant activities. They also gave better sensory attributes.

CONCLUSION: In summary, for all parameters assessed, and on a country-by-country basis, the general quality trend observed was: Ghana > Nigeria > Uganda > Kenya. All results taken together, our findings at least in part, point to the influence of geographical regions of cultivation on the quality of the ginger rhizomes.

Keywords: osmosonication; African ginger; metabolomics; sensory attributes; 6-gingerol; antioxidant activities

INTRODUCTION

The ginger rhizome is one of the most widely patronized medicinal spices and health remedy in the world. It constitutes an integral part of many traditional health systems including traditional Chinese medicine (TCM), traditional African medicine (TAM) and Ayurveda. It is a recognized remedy for nausea, motion sickness, diarrhea, etc.[1] Scientifically-proven properties include anti-inflammatory, anti-microbial, analgesic, anticarcinogenic and antioxidant effects among a host of others.[2,3] These pharmacological effects are imputed to the presence of non-volatile pungent active compounds known as the gingerols, gingerol-derivatives and essential oils.[4–6] The presence and amounts of these bioactive compounds vary depending on the varieties of ginger, climatic conditions under which they are cultivated, the area of cultivation, the harvesting season, the storage conditions, etc.[7,8] Crucial among these external factors is the impact of geographical location, since it indirectly determines the climatic and growth conditions – eventually influencing the metabolome of the matured ginger rhizomes particularly the secondary metabolites (since the primary metabolites are highly structurally conserved).[9] The secondary metabolites preserve an intricate balance with the environment, a necessary precondition for plant survival under various stress conditions.[10] Therefore by determining the levels of these metabolites in plant samples from different geographical locations invariably provides vital information about the influence

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of these locations on the chemical make-up and quality of the samples.

Quantitation of these metabolites using appropriate analytical techniques, essentially provides a point-in-time information of the activities that affect the phenotype (i.e. biochemical) of the plant under various conditions. Metabolomics therefore is the quantitation of as much as practicable, every metabolite in any biological system under distinct conditions.[11] In a previous study, we showed distinct metabolomic differences between fresh ginger rhizomes from Ghana and China.[9] The Ghana-sourced samples had higher amounts of bioactive compounds and elicited better anti-inflammatory effects.[11]

Selection of the best pretreatment and drying methods for agricultural products is very crucial. The wrong choice in this regard, could result in the deterioration of the phytochemical composition and nutritional quality of the finished product. Osmosonication (OS) works on the additive effect of osmotic dehydration and sonication resulting in benefits such as retention of bioactive compounds, preservation of medicinal qualities of the finished product, energy efficiency and short processing time.[12,13] This is a preferred pretreatment method for heat-sensitive agricultural products. We reported in a previous work that, the best pretreatment method for fresh ginger prior to drying is OS.[14] Finally, we found relative humidity convective drying as the optimal and preferred drying technique for ginger in terms of retention of the chemical integrity and quality attributes of the dried product.[15] This drying method operates on the combined influence of relative humidity and convective heat leading to the advantages stated earlier.

As part of an ongoing research on ginger, we sought to comparatively assess the quality of ginger samples from representative West African (Ghana, Nigeria) and East African (Uganda, Kenya) countries with the aim of ascertaining the best ginger on a source-dependent basis. We hypothesized that ginger rhizomes from different parts of the African continent, specifically West and East Africa would differ in their metabolomes and quality. We also aimed to investigate the probable role of geographical location on the cultivation and the quality of the ginger samples. The quality of the samples was assessed in terms of the bioactive phytochemicals composition and inherent quality attributes such as total phenolic content (TPC) and total flavonoid content (TFC), antioxidant activities, and sensory attributes. Hence samples of fresh ginger rhizomes were gotten from the representative countries, pretreated by OS, dried in a relative humidity convective dryer and the aforementioned quality characteristics determined. Finally, based on the outcome of the metabolomics evaluation, the amount of 6-gingerol in the various samples was quantitatively determined.

MATERIALS AND METHODS

Chemicals and reagents

Sodium hydroxide, sodium carbonate, catechol (*o*-diphenol), catechin, gallic acid, Folin–Ciocalteu reagent, ammonium persulfate, Sodium nitrate, potassium persulfate and hydrogen peroxide, 2, 2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1, 1-diphenyl-2-picrylhydrazyl (DPPH), copper(II) chloride, neocuproine, 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), iron(III) chloride, formic acid (HPLC grade), 6-gingerol reference standard, ammonium acetate and hydrochloric acid were bought from Sigma-Aldrich (St Louis, MO, USA) and National Pharmaceutical Corporation (Beijing, China).

Plant material

Six batches of fresh (matured) ginger rhizomes were bought from local producers in Ghana (Techiman), Nigeria (Gombe State), Kenya (Nairobi) and Uganda (Kampala) in January 2019, and taken to China after having obtained phytosanitary certificates from the various countries. They were then certified as true samples of *Zingiber officinale* Roscoe by Dr Raphael N. Alolga (a pharmacognosist) and voucher samples deposited. All samples were washed and stored briefly in a temperature-controlled room at 24 °C and humidity of 75% (during time of analysis).

Pretreatment and drying methods

The initial moisture content of the ginger samples were determined per the method of the Association of Official Analytical Chemists (AOAC, 2000) to be 80.55 ± 1.05%, 83.63 ± 2.75%, 85.78 ± 0.95%, and 86.04 ± 1.92%, (wet basis) for Ghana-, Nigeria-, Uganda- and Kenya-sourced samples respectively. It was therefore necessary to remove the possible contribution of water in these samples in subsequent determinations.

Thus, prior to drying of the ginger samples, they were pretreated by OS [20% *w/v* sucrose solution, frequency of 50 kHz, time duration (30 min), power (600 W), temperature (30 °C) and pulsed duration (10 s) on-time and (5 s) off time] as previously detailed by Osae *et al*.[14] The pretreated ginger samples (0.2 kg) were then dried in the relative humidity convective dryer (RHCD) under the following conditions; temperature (60 °C), relative humidity (20%) and air velocity (2 m s^{-1}) as earlier reported by Osae *et al*.[16]

Metabolomics study

Sample preparation

The dried pretreated ginger slices were pulverized and 200 mg of each batch weighed into 2 mL Eppendorf tubes, and 500 µL of methanol added before subjected to cold maceration for 48 h. The macerated samples were ultrasonically-extracted using an ultrasonic water bath (KQ3200DE, Kunshan, China) at 25 °C, 100 Hz for 1 h 30 min, centrifuged (using Eppendorf 5430R) at 9838 × *g* for 10 min at 4 °C and filtered through a sintered glass filter (0.22 µm). The filtrate (1 µL) of each sample was then subjected to ultrahigh-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS) analysis. An admixture of 50 µL aliquots of all samples constituted the quality control (QC) sample.

UPLC-QTOF-MS analysis

All separation conditions as well as instrumental set-up were the same as our previous report.[11] Hence, an Agilent 1290 series (Agilent Corp., Santa Clara, CA, USA) UPLC system equipped with a binary pump, micro degasser, an autosampler and a temperature-controlled column compartment was used. Separations were performed on an ACQUITY UPLC HSST3 ODS-SP column (1.8 µm, 2.1 mm × 100 mm; Waters, Dublin, Ireland).

A binary mobile phase system of 0.1% aqueous formic acid (A) and acetonitrile (B). The following gradient elution was used: 3–30% B at 0–3 min; 30–85% of B at 3–20 min; 85–100% of B at 20–23 min; 100% of B at 23–25 min; 100–3% of B at 25–27 min; 3% of B at 27–30 min. An oven temperature of 40 °C, injection volume of 0.5 µL and flow rate of 0.3 mL min^{-1} were used. Prior to each injection, the column was equilibrated for 5 min with 3% of mobile phase B. The samples were randomly injected and three injections of the QC sample was injected initially to equilibrate the column. Also, after every four injections of the samples, one

injection of the QC sample was done for the entire duration of the LC-MS run. Detection of the separated components was achieved with an Agilent 6545A QTOF mass spectrometer (Agilent Corp.) equipped with an electrospray ionization (ESI) interface. The operating parameters were as follows: drying nitrogen (N_2) gas flow rate, 8 L min^{-1}; temperature, 320 °C; nebulizer, 35 psig; capillary, 3000 V; skimmer, 65 V; OCT RFV, 750 V, fragmentor 100 V. The samples were analyzed in the positive ion mode and mass spectral data recorded across the *m/z* range 50–1000. The reference masses 121.0509 (Purine) and 922.0098 (HP-0921) were used for internal mass calibration during the runs. At the scan rate of 1.50 spectra s^{-1} using fixed collision energies (0.00, 10.00, 20.00, 30.00 V) tandem mass spectrometry (MS/MS) data were acquired with isolation width MS/MS medium (~4 amu).

Quantification of 6-gingerol

The filtrates of the extracted samples were subjected to high-performance liquid chromatography (HPLC) analysis to quantify 6-gingerol content in them. Varying standard concentrations of 6-gingerol reference were prepared in methanol and a calibration graph constructed. A Shimadzu LC-20A HPLC system equipped with a photodiode array (PDA) (Shimadzu, Kyoto, Japan) and an Inertsil ODS-SP (4.6 × 250 mm, 5 µm; GL Science Inc., Japan) were used for the quantitative analysis. The mobile phase consisted of a mixture of acetonitrile (A) and 0.1% *v/v* formic acid in water (B). The elution program was as follows: at 0–10 min, 45% of A; 10–13 min, 45–48% of A; 13–15 min, 60% of A; 15–41 min, 60% of A; 41–42 min, 67% of A; 42–44 min, 69% of A; 44–64 min, 71% of A; 64 min, 45% of A. The flow rate, detection wavelength, injection volume, and column temperature were set at 0.5 mL min^{-1}, 282 nm, 15 µL, and 30 °C, respectively. The data obtained were processed with Labsolution software (Shimadzu). This analytical procedure was duly validated according to the ICH guidelines.

Bioactive phytochemicals analysis (TPC, TFC and antioxidant activity)

Sample preparation

About 1 g amount of each powdered ginger sample was weighed and a 20 mL aliquot of 80% methanol added. This mixture was ultrasonicated for 10 min (using Trans-O-Sonic/D150-IM, Mumbai, India) and centrifuged at 10 000 × *g* for 30 min at 4 °C using Hanil, Supra 22 K (Korea). The supernatants were then used for phytochemical tests.

TPC and TFC determination

TPC and TFC of the dried ginger samples were assessed with reference to the method of Jelled, *et al.*[17] and Chen, *et al.*,[18] with minor modification. The results of TPC and TFC were expressed as milligrams of gallic acid equivalent per gram (mg GAE g^{-1}) and milligrams of catechin equivalent per gram (mg CE g^{-1}) on dry weight basis respectively. For TPC determination, Folin–Ciocalteu reagent was diluted in distilled water at a ratio of 1:10 *v/v*. Next, 1 mL of extract solution was mixed with 5 mL of Folin reagent, then, 4 mL (75 g L^{-1}) of sodium carbonate was added, vortexed for 10 min and the mixture was kept for 30 min at a temperature of 30 °C. Standard gallic acid solutions (6.25–100 mg mL^{-1} of GAE) were used to develop the standard curve (R^2 = 9979) and the reaction mixture was measured at the absorbance of 760 nm using a spectrophotometer, Model TU-1810 (Purkinje Instrument Ltd, Beijing, China). Methanol (80%) was used as the blank. The results obtained were expressed as mg GAE g^{-1} of dry weight of the sample.

With respect to TFC determination, the extracted solutions (0.5 mL) was mixed with distilled water (2 mL) and 0.15 mL sodium nitrite solution (5%, *v/v*), for 6 min. Next, 0.15 mL of aluminum chloride solution (10%, *v/v*) was added to the mixture for 6 min and 2 mL of sodium hydroxide solution (4%, *v/v*) added to the mixture. Immediately, distilled water was added up to the final volume of 5 mL and the mixture was allowed to stand for 15 min. The absorbance at 510 nm was determined by a spectrophotometer, Model TU-1810 (Purkinje Instrument Ltd). The results were calculated as mg CE g^{-1} of dry weight of the sample. The concentrations range for catechin standard solutions was 6.25 to 100 mg mL^{-1}. The R^2 of the standard curve was 0.992.

Analysis of antioxidant properties

The potential source of antioxidant properties in the dried powdered ginger samples from different geographical locations was determined based on the previous established method of Osae, *et al.*[15] and Shen *et al.*,[19] with minor modification. Assays of DPPH, ABTS, FRAP (ferric reducing antioxidant power) and CUPRAC (cupric ion reducing capacity) were measured using a spectrophotometer (Model UV-1600, Rayleigh Analytical Instrument, Beijing, China). The results were expressed as milligrams of Trolox equivalent per gram of sample on dry weight basis.

ABTS. The ABTS solution (7 mmol L^{-1}) was added to potassium persulfate (2.45 mmol L^{-1}) in a ratio of 1:1 and the resultant mixture kept in the dark at room temperature (25 °C) for 16 h. The ABTS and potassium persulfate mixture ($ABTS^+$ working solution) obtained was later mixed with 80% of methanol (*v/v*) to achieve an absorbance of 0.70 ± 0.02 at 734 nm. The ginger extract solution (145 µL) was added to 3 mL of $ABTS^+$ working solution and the mixture was stored for 25 min in the dark at room temperature and the absorbance estimated at 734 nm.

DPPH. Briefly, 0.5 mL of the extracted ginger solutions (supernatant) was added to 3 mL of methanol DPPH solution (60 mmol L^{-1}) and vortexed. The mixture was kept for 30 min at room temperature in the darkness. The absorbance (A_1) was read at 517 nm with a spectrophotometer, Model TU-1810 (Purkinje Instrument Ltd) and the blank (A_0) was run with 3 mL of DPPH solution and 0.5 mL of methanol (80%).

FRAP. Concisely, 300 µL of the supernatant was mixed with 6 mL of a solution constituted of TPTZ (10 mmol L^{-1} in 40 mmol L^{-1} hydrochloric acid), iron(III) chloride (20 mmol L^{-1}), acetate buffer (300 mmol L^{-1}, pH 3.6) in a ratio of 1:10:1 and distilled water (600 µL). The mixture was allowed to stand for 30 min at 37 °C and the absorbance was read at 593 nm. The FRAP was expressed as milligrams of Trolox equivalent per gram of sample on dry weight basis.

CUPRAC. The CUPRAC was assessed by following the method previously described by Osae *et al.*,[16] with minor modifications. The supernatant of the ginger extract (150 µL) was mixed with 4 mL of a solution constituted of neocuproine (7.5 mmol L^{-1}), copper(II) chloride (10 mmol L^{-1}), ammonium acetate (1 mol L^{-1}) and distilled water (1:1:1:1). The mixture was held for 60 min at 25 °C and the absorbance was read at 450 nm. The CUPRAC was expressed as milligrams of Trolox equivalent per gram of sample on dry weight basis.

Sensory assessment

The sensory assessment was performed based on the protocol of Kwaw *et al*.[20] Briefly, 15 assessors comprising ten males and five females were chosen from the School of Food Science and Biological Engineering, Jiangsu University. They were asked to assess the quality attributes (aroma, flavor, appearance, color and general acceptability) of the dried powdered ginger samples. Coded samples were evaluated by each of the 15-member assessors. A nine-point hedonic scale ranging from the highest (like extremely = 9) to the lowest (dislike extremely = 1) were used for the scoring.

E-nose determination of volatile compounds

The determination of the volatile (flavor) compounds of the dried ginger samples was conducted based on the method of Bonah *et al*.[21] and Osae *et al*.,[22] with minor modifications. Briefly, to measure characteristic flavor and aroma responses, the powdered samples were each placed in a 50 mL glass tube and sealed and subjected to a PEN3 electronic nose (e-nose) (AIRSENSE Analytics GmbH, Schwerin, Germany) detection. The e-nose is composed of sensor array, gas collecting system, intelligent recognition system and sensor detection curve. The sensor array system comprises ten different sensors as presented in Table 1.

Data processing and identities of metabolites ascertainment

Raw LC-MS data obtained were transformed to mzData format using DA reprocessor (Agilent) at a peak height threshold of 5000 counts. Thereafter, peak finding, filtering and alignment were achieved with R-Package XCMS and the outcome sequentially analyzed using MetaboAnalyst 4.0 as earlier reported.[9]

Graphical depictions of the different levels of the secondary metabolites in all samples were captured as orthogonal partial least squares discriminate analysis (OPLS-DA) and heatmap using R studio. The variable importance for projection (VIP) values of the OPLS-DA model were generated. The VIP values, fold change and *P*-values obtained from the *t*-test were used to establish the criterion for determining differential metabolites responsible for the observed differences between the samples. The criterion of VIP > 1, fold change >2 and *P*-value <0.05 was adopted to determine the differential metabolites. The identities of these metabolites were ascertained by a comparison of their fragmentation patterns with a reference compound and published literature.[23,24]

Table 1. Sensivities of the sensor array in PEN3 e-nose (Winmuster, Version 1.6.2, Airsense Analytics GmbH, Schwerin, Germany)

Sensors	Sensing species
S1	Aromatic organic compounds
S2	Very sensitive, broad range sensitivity, reacts to nitrogen oxides, very sensitive with a negative signal
S3	Ammonia, also used as a sensor for aromatic compounds
S4	Detect mainly hydrogen gas
S5	Alkanes, aromatic compounds, and non-polar organic compounds
S6	Sensitive to methane. A broad range of organic compounds detected
S7	Detects inorganic sulfur compounds, e.g. hydrogen sulfide. Also Sensitive to many terpenes and sulfur-containing organic compounds
S8	Detects alcohol, partially sensitive to aromatic compounds, broad range
S9	Aromatic compounds, inorganic sulfur and organic compounds
S10	Reacts to high concentrations (> 100 mg kg^{-1}) of methane and aliphatic organic compounds.

Statistical analysis

All experimental data are the results of triplicate determinations and presented as mean ± standard deviation (SD) values. The data were analyzed by one-way analysis of variance (ANOVA) with Tukey comparison test for means separation at a confidence level of 95% using Minitab v17 (Minitab Inc., State College, PA, USA).

RESULTS AND DISCUSSION

Metabolome differences between samples from the two geographical regions

The differences in the levels of the metabolites is captured in multivariate analysis shown in Fig. 1. It clearly shows the metabolomic differences with very obvious source-dependent grouping and clustering of the samples. Using chance permutation test ($n = 24$), the OPLS-DA model [R^2Y (cum) = 0.999, Q^2 (cum) = 0.659] was found to be stable and reproducible without overfitting.

Differential secondary metabolites' identities ascertainment

With earlier defined criterion as a guide, seven metabolites were identified (Tables 2 and 3). Two representations of levels of these metabolites are shown as heatmap and box plots in Figs 2 and 3 respectively. The metabolites were basically derivatives of gingerol and diarylheptanoids. Per the fold change differences as seen in Table 3, the ginger samples from West Africa generally had higher levels of the differential metabolites than those from East Africa. Based on these fold change differences, 6-gingerol levels among the samples was found to be highest. This therefore called for its accurate quantification of all samples so as to better capture the impact of the geographical location. These results reveal the impact of geographical locations and invariably different soil and climatic conditions on the quality of secondary metabolites. The outcome of this study supports previous studies in the sense that, the type and amount of secondary metabolites

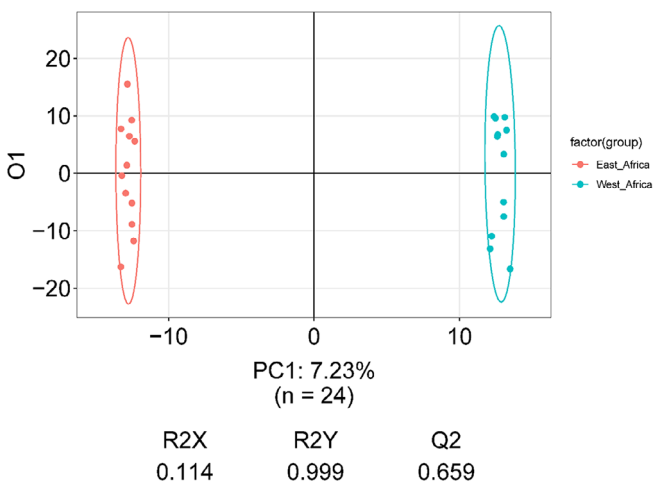

Figure 1. Supervised orthogonal partial least squares discriminate analysis (OPLS-DA) score plots of ginger samples from representative of East and West African countries. [R^2Y (cum) = 0.999, Q^2 (cum) = 0.659].

Table 2. Characteristic retention times, fragment ions and identity of differential metabolites based on comparison with reference compound and published literature

RT (min)	ID	(+)ESI-MS	(+)ESI-MS/MS	Name	Reference
6.215	M422T1123	422.2171 $[M + NH_4]^+$	207.1009,327.1586,387.1800	5-Hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-3-heptanone B	24
6.252	M438T831	438.2109 $[M + NH_4]^+$	158.9608,203.1787,277.1785	3-Acetoxy-5-hydroxy-1-(3,4-dihydroxyphenyl)-7-(3,4-dihydroxy-5-methoxyphenyl)heptane B	24
10.424	M478T629	478.2434 $[M + NH_4]^+$	461.2101,401.1947, 341.1739,163.0751	3,5-Diacetoxy-1,7-bis(4-hydroxy-3-methoxyphenyl)heptane B	24
10.71	M277T1223	277.1840 $[M + H\text{-}H_2O]^+$	177.09	6-Gingerol[a]	23
14.675	M321T69	321.2059 $[M + H\text{-}H_2O]^+$	261.1841,137.0594,163.0754	3- or 5-Acetoxy-6-gingerol A	23
15.292	M291T646	291.1589 $[M + H\text{-}H_2O]^+$	151.0749	Methyl 6-gingerol	23
18.448	M361T111	361.2740 $[M + H]^+$	137.0594,177.0907	12-Shogaol	23

[a] Compound was confirmed with reference compound.

Table 3. List of differential metabolites identified based on VIP > 1, fold change >2, *P* value < 0.05

Compound name	VIP	Fold change	*P*-Value
5-Hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-3-heptanone	1.014	2.72	0.0021
3-Acetoxy-5-hydroxy-1-(3,4-dihydroxyphenyl)-7-(3,4-dihydroxy-5-methoxyphenyl)heptane	1.297	2.98	0.0016
3,5-Diacetoxy-1,7-bis(4-hydroxy-3-methoxyphenyl)heptane	1.397	3.71	0.0087
6-Gingerol	1.436	10.34	0.0013
3- or 5-Acetoxy-6-gingerol	1.010	4.56	0.0079
Methyl 6-gingerol	1.037	3.48	0.0003
12-Shogaol	1.473	2.89	0.0001

are markedly influenced by location of cultivation, abiotic stress among a host of others.[11]

Quantification of 6-gingerol

6-Gingerol is one of the major non-volatile pungent bioactive compounds of ginger with demonstrable anti-oxidant, anti-pyretic, analgesic and anti-inflammatory activities[25–27] and a recognized chemical marker.[26] From the results of the quantitative analysis (Fig. 4, Table 4), the highest amount of 6-gingerol was recorded by the samples from Ghana 0.912 ± 0.81 μg mg^{-1}) followed by the Nigeria-sourced samples (0.814 ± 0.03 μg mg^{-1}). The samples from the representative East African countries had comparatively lower contents of 6-gingerol with those from Uganda having higher amount (0.573 ± 0.18 μg mg^{-1}) than the Kenya-sourced samples (0.42 ± 0.09 μg mg^{-1}).

TPC, TFC and antioxidant activities

A cursory examination of Table 4 reveals a consistent pattern in terms of the bioactive phytochemical make-up of the ginger samples from the four countries. As a rule of thumb, the higher the content of bioactive phytochemicals, the higher the activity in terms of TPC, TFC and antioxidant capacities. The outcome of our study proved this assertion. For all determinations, the following order was realized regardless of the phytochemical test: Ghana > Nigeria > Uganda > Kenya. For instance, the TPC analysis produced the following values: 124.90 ± 3.51 mg GAE g^{-1} for the

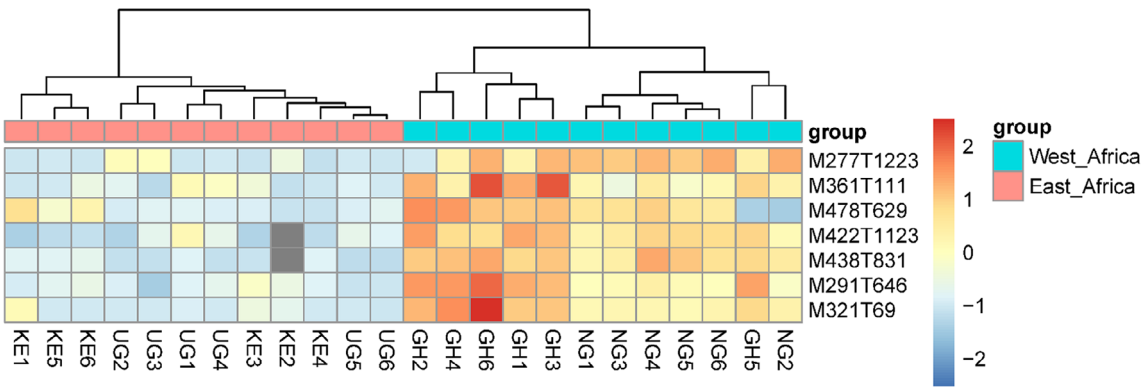

Figure 2. Heatmap representation of the levels of seven differential secondary metabolites responsible for the observed metabolomic differences in the ginger samples from the two geographical regions of Africa.

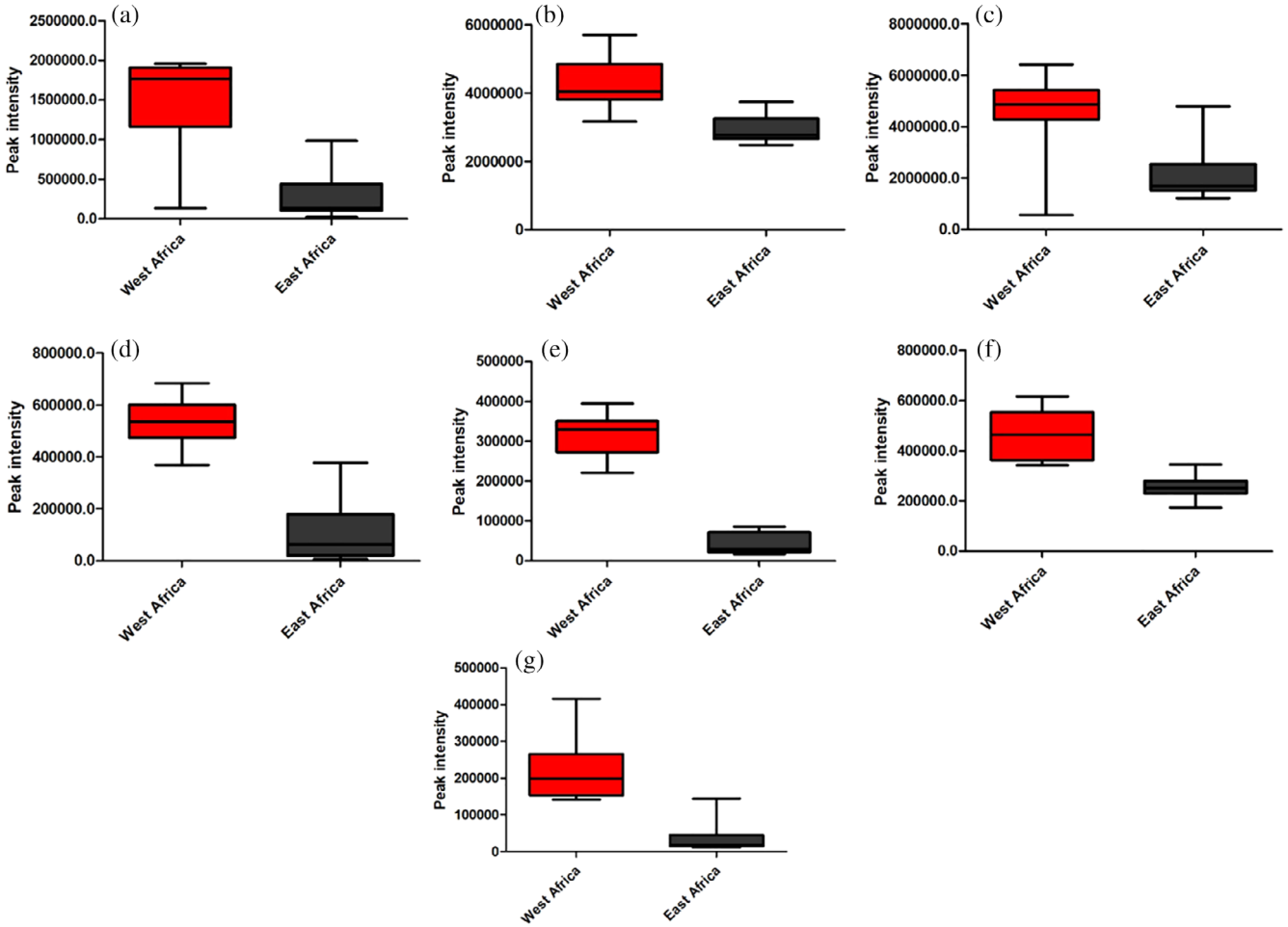

Figure 3. Box plot presentation of the relative peak intensities of seven differential secondary metabolites. (a) 5-Hydroxy-1-(4-hydroxy-3,-5-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-3-heptanone. (b) 3-Acetoxy-5-hydroxy-1-(3,4-dihydroxyphenyl)-7-(3,4-dihydroxy-5-methoxyphenyl)heptane. (c) 3,5-Diacetoxy-1,7-bis(4-hydroxy-3-methoxyphenyl)heptane. (d) 6-Gingerol. (e) 3- or 5-Acetoxy-6-gingerol. (f) Methyl 6-gingerol. (g) 12-Shogaol.

samples from Ghana, 118.23 ± 8.98 mg GAE g^{-1} for those from Nigeria, 61.70 ± 5.84 mg GAE g^{-1} for those of Ugandan origin and 53.27 ± 6.68 mg GAE g^{-1} for the ginger samples obtained from Kenya. The samples of West African origin exhibited higher antioxidant activities, TPC and TFC. These results are consistent with and corroborate those of the 6-gingerol content analysis. Holistically, these results hint on the probably influence of external conditions such as geographical region of cultivation, climate, and growth conditions on the quality of the finished products since the samples underwent the same pretreatment and drying conditions.[28–30]

Sensory evaluation of samples from the two geographical regions

Results of the sensory analysis of the ginger samples from the four countries (Ghana, Nigeria, Kenya and Uganda) as conducted by the 15 trained assessors are presented in Fig. 5. The assessors scored all the ginger samples above six on the hedonic scale. The Ghana-sourced sample was observed to have the highest score for color, appearance, aroma, flavor and general acceptance followed by the Nigeria, Uganda and with Kenya-sourced samples recording the least. The order of the sensory attributes of the samples lend support to the results of the bioactive phytochemical (i.e. TPC, TFC, antioxidant activities and 6-gingerol content) and the metabolomic analyses. These results again point to the probable contribution of the region of cultivation since the type and amount of secondary metabolites are influenced by location of cultivation, abiotic stress, etc. as earlier mentioned.[11] This glimpse of this finding was found in our previous study[15] where we reported marked physicochemical differences between dried ginger slices of Ghanaian and Chinese origin. Chen *et al.*[31] and Tchabo *et al.*,[32] also investigated the variations of antioxidant activity and sensory properties of 22 varieties of mulberry cultivars from different locations in China and established that mulberry from Zhongshen, Zhongsang, Jiangsu and Huayang locations were the best in terms of their antioxidant and sensory properties. They further reported that the difference observed among the cultivars may be due to the influence of difference growth and climatic conditions.

E-nose analysis of volatile compounds

The results the volatile (flavor) compounds assessment of the ginger samples as determined by e-nose analysis are shown in Fig. 6. The ten sensors employed showed some variations in terms of their selectivity and sensitivity. It could be observed in Fig. 6, that four sensors (S9, S5, S1, and S8) out of the ten sensors showed

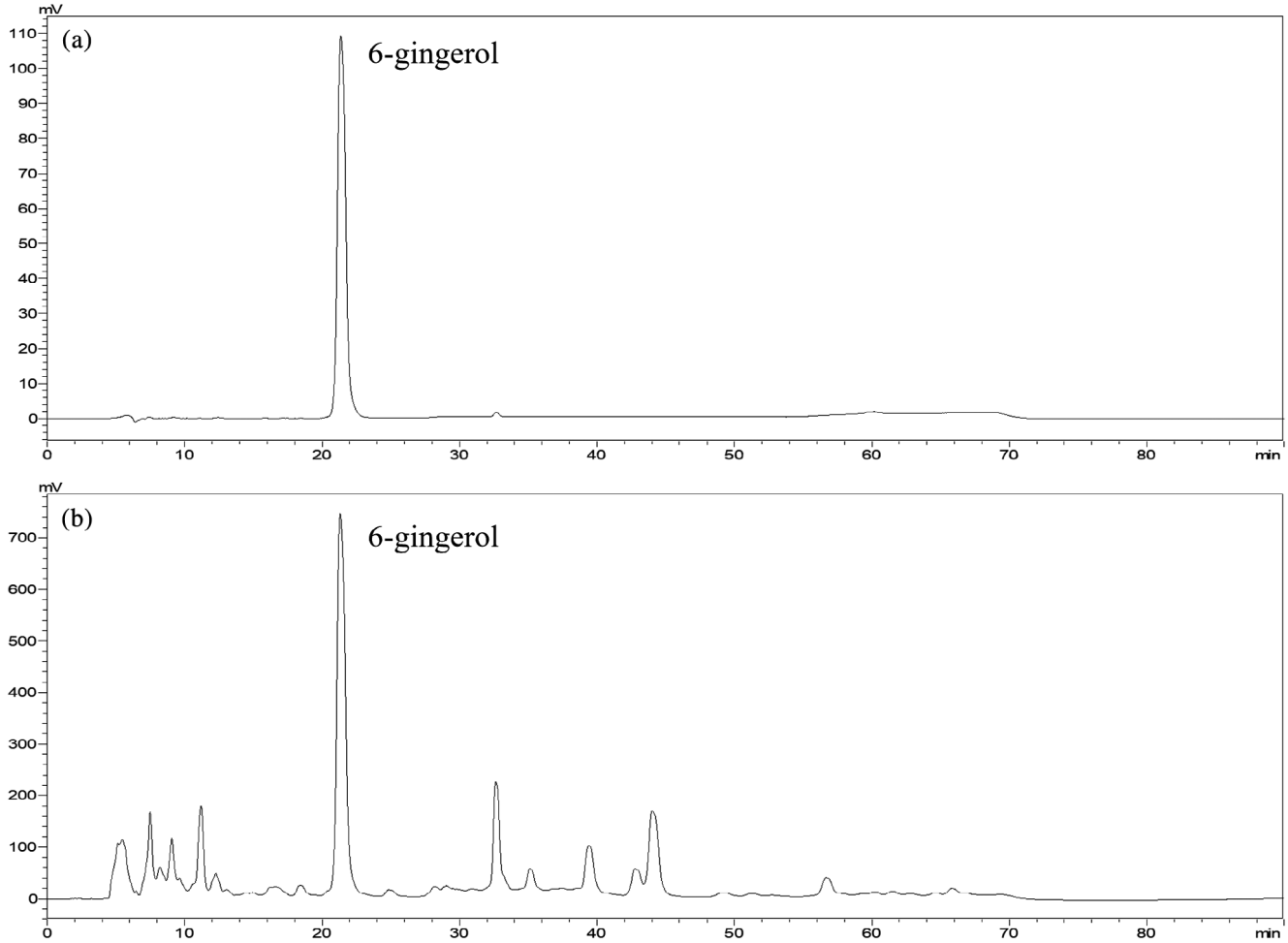

Figure 4. Typical chromatograms of 6-gingerol reference compound (a) and 6-gingerol in ginger extract (b).

higher peak values. The S9 sensor is sensitive to aromatic compounds, inorganic sulfur and organic compounds, while S5 is sensitive to alkanes, aromatic compounds, and non-polar organic compounds. S1 is sensitive to aromatic organic compounds and alcohol and S8 is partially sensitive to a broad range of aromatic compounds.

Srinivasan[1] showed that the aroma and flavor of ginger may be credited to non-volatile phenyl propanoids, derivatives of the gingerols specifically shogaols and zingerone. Shogaols and zingerone are pungent and spicy-sweet aromatic constituents of dried ginger. The volatile aromatic compounds of ginger also comprises mainly sesquiterpenoids with α-zingiberene as the key constituent constituting about 30–70%. This is in agreement with the increase in the signal intensities of S9, S5, S1, and S8 sensors which are sensitive to aromatic compounds. However, the volatile aromatic constituent was significantly higher in the Ghana-sourced sample than the other samples (Fig. 6).

Table 4. Results of 6-gingerol content, total phenolic content (TPC) and total flavonoid content (TFC) and antioxidant activities of ginger samples from selected West and East African countries

				Antioxidant activities (mgTE/g dry basis)			
Country	Content of 6-gingerol (μg mg^{-1})	TPC (mg GAE g^{-1})	TFC (mg CE g^{-1})	ABTS	CUPRIC	DPPH	FRAP
Ghana	0.912 ± 0.05 [a]	124.90 ± 3.51[a]	93.30 ± 5.05[a]	78.98 ± 6.26[a]	89.3 ± 4.95[a]	139.39 ± 7.9[a]	91.05 ± 5.52[a]
Nigeria	0.814 ± 0.03 [b]	118.23 ± 8.98[b]	83.72 ± 3.05[b]	70.5 ± 8.96[b]	84.2 ± 3.53[b]	91.82 ± 6.86[b]	78.97 ± 6.80[b]
Uganda	0.573 ± 0.01 [c]	61.70 ± 5.84[c]	55.92 ± 7.45[c]	63.4 ± 7.95[c]	76.98 ± 7.00[c]	80.48 ± 4.90[c]	70.69 ± 5.22[c]
Kenya	0.320 ± 0.09 [d]	53.27 ± 6.68[d]	44.96 ± 4.37[d]	54.06 ± 5.45[d]	65.35 ± 8.04[d]	64.4 ± 6.25[d]	62.37 ± 8.36[d]

Values followed by the different letters (a–d) in the columns are significantly different ($P < 0.05$) according to turkey test.

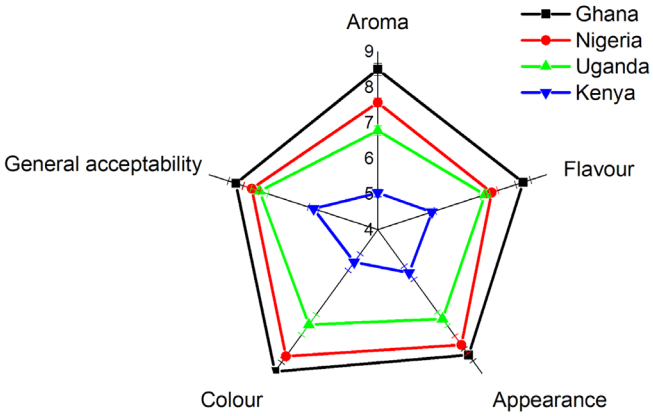

Figure 5. Sensory analysis of the ginger samples from two geographical regions.

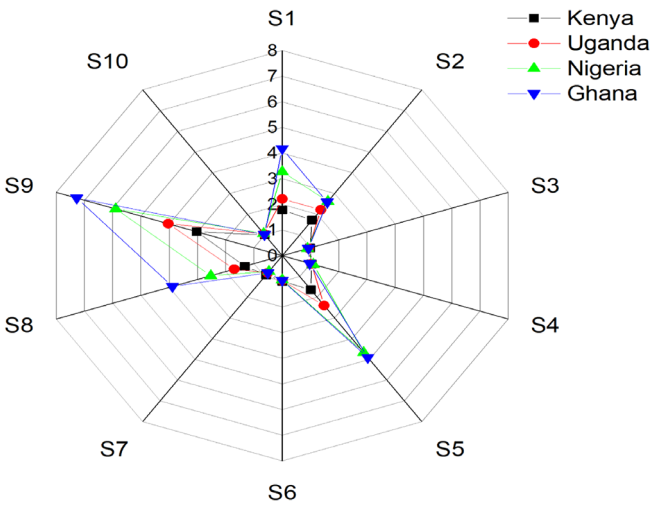

Figure 6. Radar plots of e-nose analysis of volatile compounds (flavor) of ginger samples from two geographical regions.

CONCLUSION

The outcome of our study validated our hypothesis, in the sense that, the various samples from the two geographical regions of Africa differed significantly in their bioactive phytochemical composition as clearly captured in the metabolomic analysis, TPC, TFC, antioxidant activities, 6-gingerol content, sensory attributes and levels of volatile compounds. The use of the best pretreatment (OS) and drying (RHCD) methods for the samples enabled accurate subsequent determinations. The general trend observed for all parameters determined was as follows; Ghana > Nigeria > Uganda > Kenya with the samples of West African origin being of better quality. Our study therefore clearly demonstrates at least in part, the effect of different geographical region of cultivation and the conditions inherent therein on the quality of plant samples using ginger as an example.

The main limitation of this study lies in the sample size used (i.e. number of countries and number of samples). We wish we had obtained more samples from more countries in these geographical regions or even other parts of Africa such as Southern and North Africa for comparison, but the difficulty in transporting fresh plant samples across countries limited the sample. We therefore recommend the inclusion of other countries in future studies.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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