Vacuum pressure combined with osmosonnication as an innovative pre-drying technique for Ghanaian ginger: Evidence from the metabolome and quality characteristics of the dried product

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ABSTRACT
We assessed the impact of selected pretreatment techniques, thus, vacuum-assisted osmotic dehydration (VOD), vacuum-assisted sonication (VSON) and vacuum-assisted osmosonication (VOS) on the metabolomes and quality characteristics of infrared-dried ginger slices. We found marked metabolome differences between the pretreated ginger samples, evidenced by differential amounts of 6-gingerol and 6-shogaol, total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activities. We also found distinct differences in the drying kinetics and sensory characteristics of the pretreated samples. Generally, VOS pretreatment gave the best outcomes. The VOS-pretreated samples contained the highest contents of the marker compounds, TPC, TFC and gave the best antioxidant activity. The VOS-pretreated samples also recorded the shortest drying time and exhibited the best sensory attributes. Overall, the general order observed was, VOS > VSON > VOD > control for all quality parameters examined. VOS pretreatment of ginger before drying therefore holds a great potential for large-scale industrial application.

1. Introduction

Ginger is widely known and consumed worldwide as a spice and medicinal remedy [1]. Its place in the cosmetic, pharmaceutical and food industries cannot be overemphasized [2,3]. However, the major shortfall that limits its use is its high moisture content (85 – 95% wb), thus, making it highly susceptible to microbial damage [4,5]. To this end, drying of same remains a good approach to avoid postharvest losses due to microbial degradation, enhance its quality and extend its shelf-life [6]. Conventional drying, however, has been shown to negatively influence the overall quality of the dried product. To address this fundamental limitation, various pre- and post-drying techniques have been devised with the sole aim of improving upon the final dried product quality [7,8].

Metabolomics has emerged as an attractive and useful analytical platform for all fields in the biological sciences. Its applicability in plant science and various aspects of the agricultural sciences has gained much traction in recent years. This technique basically involves the comprehensive analysis of the entire metabolites content (the metabolome) of any biological system under specific conditions. Variations in the metabolomes of plants exposed to different abiotic stress conditions help in the determination of the appropriate growth and processing conditions for same [9]. To this end, it is possible to determine the holistic impact of various postharvest conditions on the metabolome of farm produce by using metabolomics. Another approach that could be complementary to metabolomics for same purposes, is the quantification of marker compounds. Hence, a combination of these two, should provide reliable data on the quality and chemical compositions of food crops subjected to the various postharvest conditions [10].

Osmotic dehydration (OD) has attracted considerable interest in recent years for its ease of use and affordability as a pre-dehydration technique for foods. It is a relatively energy-efficient technique due to the comparatively shorter drying time it confers on treated food products which eventually leads to enhanced quality of the finished products.

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These strengths however could be fully harnessed for the purposes of improving upon the final product quality [12,13]. In line with this, a variety of innovative approaches in conjunction with OD have been investigated to improve processing efficiency and end product value [14]. OD has also been employed as a pre-dehydration technique prior to drying of certain food products including ginger [5], oyster mushroom [15] and mango [16].

Sonication technology (SON) is one of the novel techniques actively researched on as a complementary technique to OD so as to enhance processing efficiency, reduce energy consumption, and lower operational temperature. It is a green approach to various physical, technical and chemical processes [17,18]. The use of sonication technology has been reported to improve the drying rates of OD pretreated agricultural produce such garlic [18], sweet potato [19] and kiwifruit [20]. However, additional research into the impact of sonication technology on OD pretreatment is still needed due to the inconsistencies of results obtained for different crops.

Osmosonication (OS) as defined by Osae et al. [13] is the combined effect of sonication and OD. This approach is a safe and effective pre-drying technique for a variety of food crops. Its advantages includes bioactive compounds preservation, medicinal property conservation, energy efficiency and reduced processing time. Alolga et al. [18] revealed that osmosonication of Ghanaian garlic prior to drying enhanced the retention its antioxidant activity, allicin content, and led to reduced energy consumption during drying.

Vacuum technology is a new alternative pre-dehydration method that enhances the drying rate, and retention of bioactive compounds of OD-, SON- and OS- pretreated food products [21]. The reduced pressure created by vacuum enables the trapped intercellular air of agricultural crops to expand, and finally expelled by means of the pressure difference. This phenomenon leads to expansion of the external area for water diffusion and mass transfer [22]. It has been found that using vacuum at the start of the OD pretreatment enhances water loss and osmotic solute uptake in a variety of agricultural food products, including garlic [17], eggplant [21], and scallion [22].

However, as of now, there is little information regarding the combined application of vacuum pressure with SON and VOS pretreatment before drying. Therefore, the specific objectives of this study were to: (1) Assess the effects of vacuum-assisted osmotic dehydration (VOD), vacuum-assisted osmosonication (VOS) and vacuum-assisted sonication (VSON) pre-dehydration techniques on the metabolome of ginger subjected to IR drying. (2) Determine impact of same techniques on the quality of the IR-dried ginger based on the contents of two marker compounds, 6-gingerol and 6-shogaol and (3) Compare the influence of the various pretreatments on the antioxidant properties, bioactive compounds (TPC and TFC), enzyme activity (PPO), sensory (organoleptic) quality, drying kinetics and mathematical modeling of the IR-dried ginger samples. Our ultimate aim was to determine the best conditions for ginger that could have the potential for large-scale industrial application.

2. Materials and methods

2.1. Plant material

In May 2019, fresh matured ginger samples were procured from farmers in Kade, Municipal capital town of Kwaebibirem Municipal (Latitude: 6° 04’ 60.00” N Longitude: 0° 49’ 59.99”) in the Eastern Region of Ghana. The samples were transported to China after obtaining the required permit and were also verified as genuine Zingiber officinale Roscoe samples. They were thoroughly cleaned before being cut into 3–4 mm consistent slices. According to the AOAC method (2000), the fresh ginger initial moisture content was measured as 83.55 ± 1.33 % (wet weight basis).

2.2. Chemicals and reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis-(3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS), sodium hydroxide (NaOH), Folins–Ciocalteu reagent, sodium carbonate (NaCO3), catechol (o-diphenol), catechin, gallic acid, formic acid (analar grade) and acetonitrile (HPLC grade) were procured from Sigma–Aldrich, St. Louis, MO (USA). Ammonium persulfate, Sodium nitrate, Hydrogen peroxide, methanol (analar grade) and potassium persulfate were bought from National Pharmaceutical Corporation (Beijing, China). 6-gingerol reference compound (≥95% purity, CAS No. 23513–14-6), and 6-shogaol (≥95% purity, CAS No. 555–66-8) were bought from Yuanye biotechnological Ltd. Purified water was gotten from the Milli-Q water treatment system (Millipore, USA).

2.3. Pretreatment techniques

The various pretreatment (pre-dehydration) methods and their conditions are presented below.

2.3.1. Untreated (Control)

0.2 kg of the sliced ginger was soaked in water for 40 min at room temperature. After the given time, the sliced samples removed from the water were then rid of the water using absorbent tissues.

2.3.2. Vacuum-assisted osmosonication (VOS)

The modified protocol of Alolga et al. [18] was adopted. Briefly, matured fresh ginger slices (0.2 kg) were dipped in 10% (w/v) NaCl solution in an Erlenmeyer flask at a ratio of 1:10 (sample: osmotic solution), placed in a vacuum pump (Model V-100, Büchi Labortechnik AGR, Switzerland) and operated at a vacuum pressure of 100 mbar. After 20 min, the vacuum pressure was released and Erlenmeyer flask containing the ginger slices were kept in the ultrasonic bath and operated using the following: dual frequency of 20/60 kHz, time of 20 min; power of 600 W; temperature of 30 °C and pulsating period of 10 s on-time and 10 s off-time. The experiment was conducted three times and the ginger samples were taken out, drained and cleaned with tissue paper.

2.3.3. Vacuum-assisted sonication (VSON)

The VSON pretreatment was in accordance with the previously established method of Feng et al. [17] with minor alterations. Specifically, the ginger slices (0.2 kg) were dipped in sanitized water in an Erlenmeyer flask. The Erlenmeyer flask placed in a vacuum pump (Model V-100, Büchi Labortechnik AGR, Switzerland) and operated at a vacuum pressure of 100 mbar. After 20 min, the vacuum pressure was released and Erlenmeyer flask containing the ginger slices were kept in the ultrasonic bath and operated using the following: dual frequency of 20/60 kHz, time of 20 min; power of 600 W; temperature of 30 °C and pulsating period of 10 s on-time and 10 s off-time. The experiment was performed three times and the ginger samples were taken out, drained and cleaned with tissue paper.

2.3.4. Vacuum-assisted osmotic dehydration (VOD)

Previously established methodologies of Feng et al. [17] and Osae et al. [5] were employed for the VOD pretreatment with minor modifications. Briefly, the matured fresh ginger slices (0.2 kg) were dipped in 10% (w/v) NaCl solution in an Erlenmeyer flask at a ratio of 1:10 (sample: osmotic solution) for 20 min and after that it was placed in a vacuum pump (Model V-100, Büchi Labortechnik AGR, Switzerland) and operated at a vacuum pressure of 100 mbar for another 20 min. The experiment was performed three times and the ginger samples were taken out, drained and cleaned with tissue paper.
2.4. Infrared drying

With slight modifications, the pretreated ginger slices were dried in accordance with the method of Osae et al. [23]. The variously pretreated ginger slices were dried at 60 °C (temperature) with a wind speed of 2 m/s until they achieved consistent dry weights.

2.5. UPLC-Q/TOF-MS and HPLC-DAD analyses

2.5.1. Sample preparation

Four batches each of the variously dried turmeric samples were weighed (20 μg per sample). A 500 μL of methanol was added and cold macerated (4 °C) for 48 h and centrifuged at a speed of 12,000 rpm at 4 °C for 10 min. They were then filtered and the filtrates analyzed by LC-Q/TOF-MS. The quality control (QC) sample was prepared by transferring and vortex-mixing 150 μL aliquots of all samples.

2.5.2. Chromatographic analysis

The chromatographic conditions and method were the same as previously reported for ginger by Mais et al. [24]. Briefly, the separations were performed using an ACQUITY UPLC ODS column using the mobile phase composition of water with 0.1% formic acid (A) and acetonicitrile (B). The elution gradient was as follows: 3–30% B at 0–3 min; 30–85% B at 3–20 min; 85–100% B at 20–23 min; 100% B at 23–25 min; 100–3% B of B at 25–27 min; 3% of B at 27–30 min and 10 min post-run column equilibration with 3% B. An injection volume of 0.5 μL, flow rate of 0.3 mL/min and oven temperature of 40 °C were used. After every injection of the samples, the QC sample was injected twice so as to equilibrate the column while the samples were randomly injected to avoid any potential analytical biases. Same volume of the reference compounds, 6-gingerol and 6-shogaol were injected and eluted under the same chromatographic conditions. Detection of all separated compounds, 6-gingerol and 6-shogaol were injected and eluted under the same chromatographic conditions. Detections of all separated components were achieved with Agilent 6545A Q/TOF mass spectrometer (Agilent Corp., Santa Clara, CA, USA) equipped with an ESI interface. The operating parameters were as follows: drying N2 gas flow rate, 8 L/min; 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 spectra data recorded across the m/z range of 50–1000. The reference masses 121.0509 (Purine) and 922.0098 (HP-0921) were used for internal mass calibration during the runs in the positive ion mode. At the scan rate of 1.50 spectra/s using fixed collision energies (10.00, 20.00, 40.00 eV) MS/MS data were acquired with isolation width MS/MS medium (~4 amu).

2.5.3. HPLC-DAD quantification of marker compounds

Chromatographic analyses were achieved using the Dionex Ultimate 3000, Thermo Scientific UHPLC consisting of a quaternary pump, autosampler, column compartment and detector (DAD). Separations of the various components of the ginger extracts were performed through a Zorbax Extend C18 column (5 μm, 4.6 × 250 mm; Agilent, USA) with an auto-injector sampler programmed to deliver 10 μL of sample per injection. The separation was performed with a mobile phase consisting of acetonicitrile (A) and water (B) in a gradient as follows: 0–8 min, 45% B; 8–17 min, 35% B; 17–32 min, 0% B; 32–53 min, 45% B with 5 min post-run time. Total run time was 53 min, column temperature, 25 °C, flow rate, 1 mL/min and wavelength of detection, 240 nm. Data analysis and other operations were controlled using the Chromelene 7 (Chromelene Chromatography Studio).

2.6. Determination of the antioxidant activities of the variously pretreated ginger slices

Previously established procedure of Osae et al. [1] was followed for the measurement of the antioxidant activity with minor modifications. The samples were prepared by weighing 1 g of the pulverized dried slices of the variously pretreated ginger. To 1 g of the ginger samples, 20 mL of 80% methanol was added, ultrasonicated (using Trans-O-Sonic/D150-IM, Mumbai) for 10 min and centrifuged (using Hanil, Supra 22 K, Korea) at 10000 × g for 30 min at 4 °C. The supernatants were subsequently used for the antioxidant activity determinations. Four assays namely, DPPH (1, 1-diphenyl-2-picrylhydrazyl), ABTS (2, 2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), FRAP (ferric reducing antioxidant power) and CUPRAC (cupric ion reducing capacity) were used to assess the potential antioxidant activities of all ginger samples.

2.6.1. ABTS assay

ABTS solution (7 mmol L⁻¹) was added to potassium persulfate (2.45 mmol L⁻¹) at a ratio of 1:1 and the mixture stored in the dark for 16 h at room temperature (25 °C). After the specified time period, 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. A 145 μL of the extract (supernatant) of each sample was added to 3 mL of the ABTS’ working solution and the mixture was stored for 25 min in the dark at room temperature and their absorbances estimated at 734 nm using equation (1):

\[
\text{ABTS radical scavenging (\%)} = \frac{A_0 - A_1}{A_0} \times 100
\]  

where \(A_0\) is the absorbance of the solution ABTS’ solution containing the ginger samples and \(A_1\), the absorbance of the blank determined at 734 nm.

2.6.2. DPPH assay

Briefly, 0.5 mL of the extracted ginger solutions (supernatant) was added to 3 mL of methanol-DPPH solution (60 mmol L⁻¹) and vortex-mixed. The mixture was then kept for 30 min at room temperature in the dark. The absorbance (A1) was read at 517 nm with a spectrophotometer (Model TU-1810, Purkinje Instrument Ltd) and the blank absorbance (A0) was detected for 3 mL of DPPH solution and 0.5 mL of methanol (80%) and the antioxidant capacity estimated using equation (2):

\[
\text{DPPH radical scavenging (\%)} = \frac{A_0 - A_1}{A_0} \times 100
\]  

where \(A_1\) is the absorbance of the solution DPPH solution containing the ginger samples and \(A_0\), the absorbance of the blank determined at 517 nm.

2.6.3. FRAP assay

Briefly, 300 μL of the supernatant was mixed with 6 mL of a solution which was composed 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). An admixture of the ginger extracts and this solution 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.

2.6.4. CUPRAC assay

The supernatants of the ginger extracts (150 μL each) were mixed with 4 mL of a solution composed of neocuproine (7.5 mmol L⁻¹), copper(II) chloride (10 mmol L⁻¹), ammonium acetate (1 mol L⁻¹) and distilled water at volume ratio of 1:1:1:1. The resultant mixtures were left to stand for 60 min at 25 °C and their absorbances was read at 450 nm. The CUPRAC was expressed as milligrams of Trolox equivalent per gram of sample on dry weight basis.

2.7. Analysis of total phenolic (TPC) and flavonoid content (TFC)

The procedures for the assessment of TPC and TFC for the different pretreated ginger samples were same as previously reported by Alolga.
et al. [18].

For TPC analysis, 5 mL of Folin – Ciocalteu reagent diluted in water (1:10 v/v) was mixed with 1 mL of each ginger extract and 4 mL (75 g L\(^{-1}\)) of sodium carbonate. The mixtures were then vortexed for 10 min and left to stand for 30 min at 30 °C and their absorbances read at 760 nm using a spectrophotometer, Model TU-1810 (Purkinje Instrument Ltd, Beijing, China). Standard gallic acid solutions in the concentration range of 6.15–100 mg mL\(^{-1}\) were used to construct the standard curve (R\(^2\) = 0.998). Methanol (80%) served as the blank. The results obtained were expressed as milligrams of gallic acid equivalent per gram (mg GAE g\(^{-1}\)) of dry weight of the sample.

For TFC analysis, 0.5 mL aliquots of the extracted ginger solutions were each mixed with 2 mL of distilled water and 0.15 mL sodium nitrite solution (75 g L\(^{-1}\)) for 6 min and 2 mL of sodium hydroxide solution (4%, v/v) were added to the mixtures, which were allowed to stand for 30 min at 30 °C. The absorbance of each mixture was measured at 510 nm using a spectrophotometer, Model TU-1810 (Purkinje Instrument Ltd). The findings were computed as milligrams of catechin equivalent per gram (mg CE g\(^{-1}\)) of dry weight of the sample. The concentration range of 6.15 to 100 mg L\(^{-1}\) of the standard catechin solution was used and the R\(^2\) of the standard curve was 0.995.

2.8. Analysis of enzyme activity (PPO)

The PPO enzyme was extracted and the enzyme activity was measured in accordance with the method of Osaee et al. [5]. The enzyme extract solution consisting of 0.2 M sodium phosphate buffer (pH 6.5), 1% (v/v) Triton X-100 and 2% (w/v) polyvinylpyrrolidone (PVPP) was prepared. The powdered dried ginger samples were weighed (2 g of each sample), mixed with 10 mL of this solution, and the mixture homogenized and kept at 4 °C for 5 min. The mixtures were then centrifuged at 7500 × g for 30 min at 4 °C. The polyphenol oxidase (PPO) extracts (supernatants) were then immediately assessed for their enzymatic activities.

The PPO enzymatic activities of the various ginger samples were measured using a spectrophotometer Model TU-1810 (Purkinje Instrument Ltd., Beijing, China). PPO assay mixture comprising 50 μL of the ginger enzyme extract solution and 0.1 mL of catechol (0.1 M) were added to 1.95 mL sodium phosphate buffer solution (pH 6.5), vortexed for 10 s and the absorbance of same measured at 410 nm. The absorbance of the blank solution (same solution without the enzyme extract) was also measured at 410 nm. One unit of enzyme activity for PPO was defined as the amount of the enzyme which caused a change of 0.001 in absorbance unit per minute. The percent residual activity of PPO was calculated using equation (3):

\[
\text{Residual activity (\%) } = \frac{A_t}{A_{0}} \times 100
\]

where \(A_t\) is the enzyme activity of the pretreated ginger sample and \(A_0\) is the enzyme activity of the control (blank).

2.9. Sensory (organoleptic) assessment

The organoleptic properties of the ginger samples were assessed by a 30-member panel using the procedure of Kwaw et al. [25]. Briefly, 30-trained assessors comprising 15 males and 15 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. Scoring by the assessors was done using a nine point hedonic scale ranging from the highest (like extremely = 9) to the lowest (dislike extremely = 1).

2.10. Drying kinetics and mathematical modeling

Infrared drying of the fresh ginger slices was conducted at 60 °C. The drying process continued until a consistent weight was achieved. Taking cue from the work of Osae et al. [13], the moisture ratios (MR) of the variously pretreated ginger slices under infrared drying were calculated using equation (3):

\[
MR = \frac{M_r - M_e}{M_0 - M_e}
\]

Where \(M_0\) represents the starting moisture content, \(M_t\) represents the moisture content at time \(t\), \(M_e\) represents the equilibrium moisture content, and \(MR\) represents the moisture ratio. In most cases, the \(ME\) is assumed to be zero when calculating the \(MR\).

Five experimental models were used to evaluate the drying kinetics and mathematical modeling of the ginger slices (i.e., Weibull, Wang & Singh, Modified Page, Lewis and Henderson & Pabis) are presented in Table 1.

The optimal models that suitably described the drying curves of the variously pretreated ginger samples were then determined using mathematical models. RMSE (root mean square error), RSS (residual sum of squares), \(x^2\) (reduced chi-square) and \(R^2\) (Coefficient of determination) were computed using the underlisted equations (equations (5), 6 and 7) and applied to assess the appropriateness of the models.

\[
x^2 = \frac{\sum_{i=1}^{N} (MR_{exp} - MR_{exp,i})^2}{N - \zeta}
\]

\[
RSS = \sum_{i=1}^{N} (MR_{exp} - MR_{exp,i})^2
\]

\[
R^2 = 1 - \frac{\sum_{i=1}^{N} (MR_{exp} - MR_{exp,i})^2}{\sum_{i=1}^{N} (MR_{exp} - MR_{exp})^2}
\]

2.11. Metabolomic data processing and other statistical analyses

The raw LC-MS data files were converted to the mzData format using a peak height threshold of 5000 count with the DA reprocessor (Agilent). These subsequent activities were achieved using the free to access R-package XCMS, thus, peak finding, filtering and alignment. Principal component analysis (PCA), partial least squares discriminate analysis (PLS-DA), and heatmap were constructed with R studio (3.6.1). Tripli-cate determined data were presented as mean ± standard deviations while means of all analyses were compared using the Tukey test (Minitab Inc., Pennsylvania, USA). Statistically significant values were those with \(P\) values < 0.05. P-values of < 0.05, 0.01, and 0.001 were considered statistically significant, very significant, and extremely highly significant, respectively. Analysis of rehydration kinetics data were done using the 2018 version of the OriginPro Software.

### Table 1

| Model name           | Equation             | Reference |
|----------------------|----------------------|-----------|
| Modified page        | \( MR - \exp(-kt^n) \) | [6]       |
| Weibull              | \( MR - \exp\left(-\left(\frac{t}{\beta}\right)^{\alpha}\right) \) | [13]      |
| Wang and Singh       | \( MR - a + bt + ct^2 \) | [3]       |
| Henderson and Pabis  | \( MR - \exp(-kt) \)  | [25]      |
| Lewis                | \( MR - \exp(-kt) \)  | [36]      |
3. Results and discussion

3.1. Distinct metabolomes between the different pretreated ginger samples

Figure 1 shows characteristic total ion chromatograms (TIC) of the different pretreated ginger samples. The TIC shows slight parallels in the heights of the peaks between CTL, VOS, and VOD, with the sole variance being the VSON sample. An analysis of the chromatograms reveals minute differences in the metabolites content of the variously pretreated samples. This minor distinction, however, was important enough to be captured during the analysis. To determine if these similarities and minor differences were statistically significant, the retrieved LC-MS data were submitted to unsupervised principal component analysis (PCA), supervised partial least squares discriminate analysis (PLS-DA) and heatmap-clustering analysis. In contrast to visual inspection, the findings of the analyses revealed highly distinct differences between the variously pretreated ginger samples (Fig. 2). The samples are clearly clustered and grouped according to the various pre-drying methods. Fig. 2A depicts the results of unsupervised PCA analysis, whereas Fig. 2B depicts the PLS-DA algorithm for all 16 samples. PCA is an unsupervised model that considers all variables whereas PLS-DA, which is a supervised model excludes systemic noise and only considers information on relevant variables. As a result, the PLS-DA model outperforms the PCA model in classification efficiency. Using the PLS-DA model (Fig. 2B), an unequivocal distinction was achieved for the variously pretreated ginger samples evidenced by their clustering in the different quadrants. The $R^2$(cum) = 0.987 and $Q^2$(cum) = 0.962 values, show that the PLS-DA model has a high degree of fitness and predictability. These differences in metabolites’ levels of the various pretreated ginger samples are pictorially summarized as heatmap (Fig. 2C).

Using a selection criterion of fold change (FC) $>$ 2, variable importance in projection (VIP) $>$ 1 and p-value $<$ 0.05, we determined 5 metabolites to be differentially abundant in the ginger samples subjected to the various pretreatments, known as differential secondary metabolites (Tables 2 and 3). These compounds were identified based on their characteristic fragmentation patterns with reference to published literature and reference compounds (Table 2). Based on the FC of these 5 compounds, 6-gingerol and 6-shogaol had high values of 9.7812 and 5.7215 respectively (Table 3). These differential metabolites are frequently used as chemical markers to characterize the quality of ginger samples and were therefore considered for subsequent HPLC quantification.

Taken together, the outcome of the metabolomics study holistically captured the impact of the different pretreatments on the metabolite profiles of the ginger samples. It also provides a proof-of-concept on the utility this approach (untargeted metabolomics) in the food industry in respect of determining the impact of various post-harvest processing techniques on the quality of the finished product, in this case, ginger. It could therefore be a helpful tool in the selection of the appropriate processing technique as reported by other researchers for other plant material such as black raspberry [26], and Salvia miltiorrhiza Bunge [27,28]. The impact of the various pretreatments on specific quality parameters of the ginger samples are discussed below.

3.2. Contents of marker compounds in the different pretreated ginger samples

To better capture the effect of the various pretreatment techniques on the quality of the finished (dried) ginger products, the marker compounds, 6-gingerol and 6-shogaol, on the basis of their high FC values as captured in the untargeted metabolomics analysis, were quantified by HPLC-DAD analysis. 6-gingerol and 6-shogaol are two of the major non-volatile pungent bioactive compounds with known anti-oxidant, anti-pyretic, analgesic and anti-inflammatory activities and considered as markers of ginger quality. The former is reported to be more abundant in fresh ginger while the converse is true for the latter for dried ginger [23]. The levels of these two compounds could therefore give a clear indication of the impact of different pre-drying treatments on the quality traits of the dried ginger. The identities of these marker compounds in the samples were ascertained on the basis of their retention times relative to reference compounds. The average retention time for 6-gingerol was 5.973 min while that for 6-shogaol was 47.490 min (Fig. 3). The equations of the calibration graphs and regression coefficients ($R^2$) for 6-gingerol and 6-shogaol were respectively: $y = 29.958x + 0.8927$, $R^2 = 0.9946$; $y = 228.12x + 46.442$, $R^2 = 0.9953$. Table 4 summarizes the outcome of our analysis. The highest 6-gingerol content was found in the VOS pretreated samples (7.922 ± 0.076 μg/mg) while the control samples had the lowest content of same. The VSON and VOD pretreated samples respectively contained 5.779 ± 0.045 and 5.721 ± 0.030 μg/mg of 6-gingerol.

A similar trend was realized for the content of 6-shogaol for the variously pretreated ginger samples albeit with relatively lower values. From these results, it is clear that treatment of the ginger slices by VOS before drying was the superior technique compared to the rest. This pretreatment method essentially preserved the highest 6-gingerol content after drying. The most plausible explanation for the observed differences stems from the modes of operation of the various pretreatment techniques. VOD operates on the influence of vacuum pressure and
osmotic dehydration (V + OD). VSON is based on the combined effects of vacuum pressure and sonication (V + US). However, VOS pretreatment operates on the combined effects of vacuum pressure, sonication and osmotic dehydration (V + US + OD). The additive effects of these three parameters probably resulted in increased cell wall disruption and greater mass transfer, resulting in the enhanced release and/or extractability of the marker compounds. Similarly, the influence of vacuum pressure and the acoustic cavitation of sonication (in the case of

Fig. 2. Pictorial depictions of the metabolome differences between the variously pretreated ginger samples. (A). Unsupervised principal component analysis, PCA (B). Supervised partial least squares discriminant analysis, PLS-DA (C). Heatmap depiction of holistic metabolites difference.

Table 2
Details of the five differential metabolites (potential marker compounds) identified in the various pretreated ginger samples.

| Tentative assignment | Formula   | Cal. m/z [M + H]⁺ | Det.m/z [M + H]⁺ | Δppm    | MS/MS fragmentation | Reference |
|----------------------|-----------|-------------------|-------------------|---------|---------------------|-----------|
| 4-gingerol           | C13H22O4  | 267.1591          | 267.1589          | 0.7486  | 249.1086, 177.0551  | [8]       |
| 6-gingerol*          | C17H26O4  | 295.1904          | 295.1910          | 2.0326  | 277.1846, 177.0916  | [8]       |
| 8-gingerol           | C19H30O4  | 323.2217          | 323.2212          | 1.5469  | 305.1125, 177.0548  | [8]       |
| 10-gingerol          | C21H34O4  | 351.2530          | 351.2526          | 1.1388  | 333.2421, 177.0550  | [8]       |
| 6-shogaol*           | C17H24O3  | 277.1798          | 277.1807          | 3.2469  | 177.0911, 137.0599  | [8]       |

*Confirmed with reference compounds.
samples respectively. The FRAP assay also produced the following results for the VOS, VSON, VOD and the control (untreated) dried ginger samples: 101.70, 85.71, 66.03 and 48.66 mgTE/g db. These outcomes are agreeable with Feng, et al. [17] as they proved more effective in the release and/or extractability of these compounds than the additive effects of vacuum pressure and the osmotic dehydration pressure gradient for VOD. The ability of any pretreatment method to enhance the release and/or extractability of the active compounds of any food crop or plant produce is thus crucial and should be given utmost priority. This is a view that is equally held by other researchers for a variety of plant produce [17,18].

3.3. Antioxidant activities of the variously pretreated ginger slices

The restriction or prevention of nutritional oxidation (especially of lipids and proteins) through oxidative chain reactions is termed as antioxidant activity. The methods commonly used to assess this activity include the ABTS, CUPRAC, DPPH and FRAP methods. The impact of the various pre-dehydration techniques (VOD, VSON and VOS) and the control on the antioxidant activities of the dried samples is depicted in Table 4. All the pre-dehydration techniques significantly (P < 0.05) enhanced the retention of the antioxidant activities of the ginger samples than the untreated (control) sample. The results of the ABTS assay were 67.2, 49.97, 38.50 and 23.01 mgTE/g db for VOS, VSON, VOD and the control respectively. The DPPH antioxidant capacities of the VOS, VSON, VOD and the control dried ginger samples were respectively 132.26, 112.95, 99.91 and 64.86 mgTE/g db. The outcome of the CUPRAC assay was as follows: 101.70, 85.71, 66.03 and 48.66 mgTE/g db for the VOS, VSON, VOD and the control (untreated) dried ginger samples respectively. The FRAP assay also produced the following results: 93.23, 74.22, 50.22 and 36.40 mgTE/g db for the VOS, VSON, VOD and the control (untreated) dried ginger samples respectively. The results of all the assays followed a consistent pattern of VOS > VSON > VOD > Untreated ginger samples. The maximum antioxidant activities of the VOS-pretreated samples can be attributed to the synergy of the vacuum pressure, sonication and osmotic dehydration. The combined effects of these three processes resulted in increased cell wall disruption and extractability, as well as greater mass transfer, leading to the extra release of phenolic compounds [22]. These outcomes are agreeable with Feng, et al.[17] as they reported enhanced antioxidant activities for VOS-pretreated garlic slices prior to drying. Secondly, the high antioxidant activities of the VOS-pretreated samples may be credited to the relatively shorter drying time of these samples relative to the other pretreated samples, thus, preserving a greater proportion of same. This finding is consistent with the earlier report of Vieira da Silva Júnior et al. [29]. Similarly, we also discovered a relationship between antioxidant activities and TPC, implying that the high antioxidant activity could be attributed in part to its increased TPC. According to Alolga et al. [18], treatment of Ghanaian garlic by VOS and sonication accelerates the production of free radicals, improves phenolic compounds polymerization, and advances

Table 4

| Pretreatment method | Content of marker compound (mg/g sample) |
|---------------------|-----------------------------------------|
|                     | 6-gingerol | 6-shogaol |
| CTL                 | 4.021 ± 0.028 | 2.441 ± 0.017 |
| VOD                 | 5.721 ± 0.030 | 2.953 ± 0.014 |
| VSON                | 5.779 ± 0.045 | 3.072 ± 0.010 |
| VOS                 | 7.922 ± 0.076 | 3.109 ± 0.012 |

C TL, Control (untreated); VOS, Vacuum-assisted osmosonication; VSON, Vacuum-assisted sonication; VOD, Vacuum-assisted osmotic dehydration.

Table 5

| Drying methods | Antioxidant activities (mgTE/g db) | Total phenolic (mg GAE/gdw) | Total flavonoid (mg CE/gdw) |
|----------------|-----------------------------------|-----------------------------|----------------------------|
|                | ABTS                             | CUPRAC                      | DPPH                       | FRAP | TPC          | TPC          |
| VOS            | 67.2 ± 2.08 ± 3.14 ± 3.14 ± 1.64 | 93.23 ± 3.14 ± 171.81       | 98.78                       |
| VSON           | 49.97 ± 0.84 ± 2.16 ± 2.85       | 74.22 ± 1.95 ± 100.59       | 80.44                       |
| VOD            | 38.50 ± 1.82 ± 0.54 ± 0.54       | 50.22 ± 0.82 ± 83.73        | 69.13                       |
| CONTROL        | 23.01 ± 2.00 ± 3.40 ± 3.40       | 36.40 ± 1.43 ± 65.91        | 53.47                       |

VOS, Vacuum assisted osmosonication; VSON, Vacuum assisted sonication; VOD, Vacuum assisted osmotic dehydration; CONTROL, untreated; Difference values followed by the different letters (a-f) in the column are significantly different (P < 0.05) according to tukey test.

VIP, variable importance in projection.
antioxidant capabilities. Similarly, Feng et al. [17], discovered that pretreating Chinese garlic slices by VOD and VSON before drying improved their quality.

3.4. TPC and TFC of the variously pretreated ginger

According to Galanakis and Kotsiou [30], bioactive substances are phytochemicals that can be isolated from foods or food waste that have the ability to control metabolic functions, resulting in positive outcomes. Table 5 is the summary of the results of TPC and TFC (bioactive compounds) of the samples pretreated by VOS, VSON, and VOD as well as the control (untreated). Regardless of the bioactive phytochemicals test for TPC and TFC, the same trend was realized for all samples: VOS > VSON > VOD > Control dried ginger samples. The results of the TPC analysis were: 117.81, 100.59, 83.75 and 65.91 mg GAE/g dw for VOS, VSON, VOD and the control respectively. Also, the results of the TFC were 98.78, 80.44, 69.13 and 53.47 mg CE/g dw for VOS, VSON, VOD and the control samples in that order. From these outcomes, it is evident that the VOS-pretreated dried ginger samples significantly (P < 0.05) recorded the highest content of TPC and TFC than those pretreated by the other samples. These results might be ascribed to the synergistic impact of OD pressure gradient, the vacuum pressure and the acoustic cavitation of sonication which compromised the cell structural integrity of the ginger slices resulting in the formation of numerous microchannels on and within the surfaces of same [18]. This mechanism resulted in enhanced diffusion of water from the ginger samples, enhanced the speed of dehydration, thus, accounting for the short drying time and the high TFC and TPC (bioactive compounds) compared to the other pretreatment techniques (VSON and VOD). Wang et al. [22] found a similar result when scallion was treated by VOS prior to drying. The application of VSON and VOD pretreatment procedures preserved a greater amount of the TPC and TFC than control ginger samples. The probable reason for this observation is same as earlier outlined for the antioxidant activities of same.

3.5. Enzyme inactivation of the different pretreated ginger slices

One of the main goals of using food processors is to decrease enzyme activity, as an increased enzyme activity in food products can contribute to product deterioration. One of the factors that account for increased antioxidant activity of food crops is the deactivation of endogenous oxidative enzymes after drying. The result of the PPO enzyme activity of the dried ginger samples variously treated is depicted in Fig. 4. The dried VOS-pretreated ginger samples recorded the lowest residual enzyme activity (REA) relative to the other samples. The possible explanation might be credited to the influence of cavitation by sonication waves, osmotic pressure and the impact of vacuum pressure which collectively caused unfavorable circumstances for enzymatic action. Feng et al. [17] reported similar findings for Chinese garlic prior to drying. Sango et al. [31] who studied the effect of vacuum pressure and sonication on the enzymatic inactivation of blackberry juice established that the combined impact of ultrasonic cavitation and vacuum pressure, that culminated in high temperature and pressure, resulting in enzymatic structure breakdown, is the probable cause of the decreased enzyme activity of VSON-pretreated samples. Lastly, the VOD pretreatment was more effective than the control (untreated) at inactivating the PPO enzymes, as shown in Fig. 4. This is might be due to the synergistic impact of the sonication cavitation which chemically and mechanically influences the enzyme system as well as the inherent high osmotic pressure leading to the break down of same. de Jesus Junqueira et al. [32], who investigated the impact of VOD on the quality of several vegetables reported similar findings for the PPO enzyme inactivation in carrot, eggplant and beetroot.

3.6. Sensory analysis of the different pretreated dried ginger slices

The outcome of the sensory assessment of the dried ginger pretreated by VOS, VSON, VOD and the control (untreated) as performed by the 30-trained panelist is depicted in Fig. 5. On a 9-point hedonic scale, the assessors gave all the three pre-dehydration procedures (VOS, VSON, and VOD) a score of at least 6.4. The dried VOS-pretreated ginger was adjudged to have the maximum score as regards its appearance, flavor, color, aroma, and general acceptability followed by the VSON- pretreated samples while the control (untreated) ginger sample received the lowest score. The general trend of the variously pretreated samples in terms of the antioxidant activity, TPC, TFC, and enzyme activity are given credence by their sensory attributes (i.e., VOS > VSON > VOD and Control). The probable reasons for these results have already been outlined in the earlier sections. Lagnika et al. [33], who studied the impact of different pretreatments (VSON and VOD) of sweet potato prior to microwave drying attributed the high sensory properties and other quality properties of same to the joint influence of sonication, osmotic dehydration and vacuum pressure. According Dias da Silva et al. [34], VOS pretreatment of melon preceding drying enhanced its drying efficiency and produced finished products with good and acceptable sensory qualities.

Fig. 4. Enzyme inactivation of dried ginger slices under various pretreatment conditions.

Fig. 5. Sensory analysis of the dried products of different pretreated ginger slices.
3.7. Drying kinetics and mathematical modeling of drying curves

Figure 6 shows the graphical depiction of the drying kinetics (moisture ratio vs. drying time) of the variously pretreated ginger (VOS, VSON, VOD) and the control (untreated) samples. With reference to the untreated (control) sample, all of the pre-dehydration procedures enhanced the drying of the samples. The drying times for the VOS-, VSON-, VOD-pretreated ginger samples and the control were 4, 6, 7, and 9.5 h respectively. These findings show the various pre-dehydration strategies reduced the drying time by 42.11%, 63.16 % and 73.68 % for VOS, VSON and VOD respectively. The possible reasons that account for this difference are same as earlier outlined. According to Alolga et al. [18], VOS pretreatment of Ghanaian garlic before drying reduced the drying time by 57.90%. They attributed the shortened drying time to the creation of numerous micropores on the garlic cell structure which facilitated the exit of moisture. Feng et al. [17] and Wang et al. [22] reported similar findings for the drying of Chinese garlic and scallion.

Five empirical models, Weibull, Wang & Singh, Modified Page, Lewis, and Henderson & Pabis were chosen to estimate the drying kinetics of the variously pretreated ginger samples (Table 6). The following statistical metrics were used to forecast the suitability of each model for the experimental data: coefficients of determination (R²), root mean square error (RMSE), root sum of squares (RSS), and Chi square (χ²). The models with the highest R² and the lowest RMSE, RSS and χ² values were adjudged the most suitable. As summarized in Table 6, the Modified Page Model gave the highest values for R² and the lowest values for RMSE, RSS, and χ² for our experimental data, hence, was consequently, deemed the best model. Nguyen et al. [35] and Doyraz [36], reported the Modified Page and Weibull models as the appropriate models for the drying of lemon grass and persimmon slices respectively.

4. Conclusions

With respect to the various investigations conducted in this study, the following conclusions can be arrived at: (1) The various pre-dehydration (pretreatment) techniques affected the entire metabolomes of the ginger slices to varying degrees as captured by the metabolomics analysis. (2) These metabolome differences were typified by the differential levels of the marker compounds in the various pretreated ginger samples. (3) The metabolic differences of the various pretreated ginger samples were also evident in the differential antioxidant activities, TPC, TFC, enzyme activities and sensory qualities. (4) The various pretreatment techniques affected the microstructure of the ginger slices to varying degrees, thus, accounting for differences in their drying kinetics. (5) VOS pretreatment gave the best results for the quality parameters examined. The general trend observed for all the determinations was VOS > VSON > VOD > Control. VOS pretreatment prior to drying thus holds a huge potential for large-scale industrial application in the production dried ginger of high quality.

CRediT authorship contribution statement

Raphael N. Alolga: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. Richard Osae: Conceptualization, Investigation, Methodology, Software, Supervision, Validation, Writing – original draft. Maurice T. Apaliga: Methodology, Software, Writing – review & editing. Traore S. Ibrahim: Investigation, Resources. Mohammed S.A. Ahmed: Investigation, Software, Validation, Writing – review & editing. Emmanuel Kwaw: Eric A. Antiri: Methodology, Resources, Software, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

Table 6

| Pretreatment method | Model name | R² | χ² | RSS | RMSE |
|---------------------|------------|----|----|-----|------|
| Control             | Modified page | 0.98893 | 3.2949 × 10⁻³ | 0.00168 | 0.00966 |
|                     | Weibull     | 0.99892 | 8.9124 × 10⁻⁵ | 0.00425 | 0.01742 |
|                     | Wang and Singh | 0.94765 | 0.00454 | 0.08179 | 0.06741 |
|                     | Henderson and Pabis | 0.99892 | 9.3539 × 10⁻⁵ | 0.00169 | 0.00969 |
|                     | Lewis       | 93.32 | 0.11451 | 2.06117 | 0.33839 |
| VOD                 | Modified page | 0.99671 | 3.1247 × 10⁻⁴ | 0.00407 | 0.01768 |
|                     | Weibull     | 0.99656 | 3.03314 × 10⁻⁵ | 0.00425 | 0.01742 |
|                     | Wang and Singh | 0.94926 | 0.00482 | 0.06265 | 0.06942 |
|                     | Henderson and Pabis | 0.9967 | 3.1379 × 10⁻⁵ | 0.00407 | 0.0177 |
|                     | Lewis       | 96.66 | 0.10491 | 1.25892 | 0.3239 |
| VSON                | Modified page | 0.99809 | 1.94874 × 10⁻⁴ | 0.00214 | 0.01396 |
|                     | Weibull     | 0.99799 | 1.88023 × 10⁻⁴ | 0.00226 | 0.01371 |
|                     | Wang and Singh | 0.95111 | 0.00499 | 0.05484 | 0.07061 |
|                     | Henderson and Pabis | 0.99806 | 1.98069 × 10⁻⁴ | 0.00218 | 0.01407 |
|                     | Lewis       | 92.20 | 0.10716 | 1.17877 | 0.32735 |
| VOS                 | Modified page | 0.99761 | 2.50977 × 10⁻⁵ | 0.00251 | 0.01584 |
|                     | Weibull     | 0.9971 | 2.9927 × 10⁻⁴ | 0.00239 | 0.0173 |
|                     | Wang and Singh | 0.94054 | 0.00624 | 0.0624 | 0.07899 |
|                     | Henderson and Pabis | 0.99717 | 2.70024 × 10⁻⁴ | 0.0027 | 0.01643 |
|                     | Lewis       | 93.24 | 0.11813 | 0.94502 | 0.3237 |

VOS, Vacuum assisted osmosonication; VSON, Vacuum assisted sonication; VOD, Vacuum assisted osmotic dehydration; Control, untreated.
the work reported in this paper.

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