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Sonication, osmosonication and vacuum-assisted osmosonication pretreatment of Ghanaian garlic slices: Effect on physicochemical properties and quality characteristics

Raphael N. Alolga [a,*], Richard Osae [b], Gloria Essilfie [c,*], Firibu Kwasi Saalia [d], Selorm Akaba [e], Fadzai Chikari [b]

[a] State Key Laboratory of Natural Medicines, Department of Pharmacognosy, China Pharmaceutical University, No. 639 Longmian Road, Nanjing 211198, China
[b] School of Applied Sciences and Technology, Department of Food Science and Postharvest Technology, Cape Coast Technical University, Cape Coast, Ghana
[c] College of Basic and Applied Sciences, School of Agriculture, Department of Crop Science, University of Ghana, Ghana
[d] College of Basic and Applied Sciences, School of Engineering, Department of Food Process Engineering, University of Ghana, Ghana
[e] College of Agriculture and Natural Sciences, School of Agriculture, Department of Agricultural Economics and Extension, University of Cape Coast, Cape Coast, Ghana

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ABSTRACT

This work aimed to assess the impact of sonication (US), osmosonication (US + OD) and vacuum-assisted osmosonication (V + US + OD) pretreatments on the quality of Ghanaian garlic prior to relative humidity convective drying. Hence, slices of fresh garlic subjected to US, US + OD and V + US + OD pretreatments were dried and the following assessed: antioxidant activities, total phenolic content (TPC), total flavonoid content (TFC), polyphenol oxidase (PPO) enzyme inactivation, rehydration ratio (RR), drying kinetics, energy consumption, chromatographic fingerprinting, allicin content and Fourier-transform infrared (FT-IR) spectroscopy. V + US + OD pretreatment gave the best results for antioxidant activities, TPC, TFC, RR and PPO enzyme inactivation. It also recorded the shortest drying time and was more energy efficient. Finally V + US + OD pretreatment maintained the chemical integrity of the finished product and recorded the highest content of allicin. A general trend was however observed for all quality parameters assessed for the various pretreatment methods as: V + US + OD > US + OD > US.

1. Introduction

Garlic remains among the upper echelons of spices in the culinary and food industries. It also maintains a credible position in traditional medicinal remedies world wide for the treatment of a plethora of diseases (Feng et al., 2018). Scientific research has established its medicinal potential in decreasing blood pressure and cholesterol levels, boosting immunity and improving cardiac function among a host of others (Hussain, Ali, Miraj, & Sajjad, 2014). Much of these pharmacological properties are credited to the organosulphur compounds with allicin being the principal bioactive compound. The amount of allicin (Diallyl thiosulphate) forms the basis for the quality assessment and standardization of garlic-based products. For instance, the British Pharmacopoeia recommends a minimum of 4.5 mg/g of allicin in garlic products meant for pharmaceutical and economic purposes (Khar, Banerjee, Jadhav, & Lawande, 2011). The high moisture content of fresh garlic (65–75% wet basis) makes it very prone to quality loss and deterioration by microorganisms. Hence, most commercial garlic-based products are available as dried powders.

Drying remains a good method for the preservation of garlic. It reduces the moisture content, extends the shelf-life and reduces the bulk of the finished product (Bozkir, Ergün, Tekgül, & Baysal, 2019). However, conventional drying is reported to negatively impact on the quality of the final product (Wang et al., 2017). To circumvent this challenge, pretreatment of fresh samples prior to drying emerges as a viable approach. A pretreatment method that will reduce the moisture content and ultimately conserve the medicinal and nutritional quality of the finished product is worth adopting.

Sonication (US) is a known pre-dehydration (pretreatment) method. It is the energy from sound waves with frequency above 16 kHz. The use of US results in a sponge-like effect, as well as mechanical and chemical cavitation, thereby creating microscopic pores and micro channels on

* Corresponding authors.
E-mail addresses: alolgara@cpu.edu.cn (R.N. Alolga), gessilfie@ug.edu.gh (G. Essilfie).

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the surface of the food sample and enhancing mass transfer (Mothibe, Zhang, Nsor-atindana, & Wang, 2011)). This results in enhanced drying efficiency, reduced drying time and energy consumption, and retains product quality. (Amami et al., 2017). It has been used prior to the dehydration of various agricultural products including bitter melon (Jin, Zhang, & Shi, 2019), strawberry, and ginger (Osae, Zhou, Xu, Tchabo, Tahir, Mustapha et al., 2019). However, previous studies have sought to bring out the potential benefits of combining other pretreatment methods with US. The synergism between US and osmotic dehydration (Çağlayan & Barutçu Mazı, 2018), high pressure (Bello, Martínez, Ceberio, Rodrigo, & López, 2014), vacuum pressure (Wang et al., 2019), pulsed electric field (Kumar, Patel, & Kumar, 2015), cold plasma (Zhang et al., 2019) etc. enhances its efficiency.

Osmosonication (US + OD), which operates under the combined influence of osmotic dehydration and sonication is a credible pretreatment method for most agricultural products (Osae et al., 2019b). Its strengths include retention of bioactive compounds, preservation of medicinal properties, energy efficiency, cost effectiveness and shortening of processing time (Amami et al., 2017; Çağlayan & Barutçu Mazı, 2018). Amami et al. (2017) also showed that osmosonication pretreatment of strawberries resulted in preserving the bioactive compounds, antioxidant activity, and phenolic content of the final product. Osae et al., (2019a) established that US + OD pretreatment led to the retention of the bioactive compounds (gingerols and their derivatives), inactivation of polyphenol oxidase and peroxidase enzymes and enhancement the antioxidant activity of Ghanaian ginger.

Although US and US + OD have been used as pretreatment methods prior to drying of different agricultural products, to our knowledge, there is still limited information on the additive influence of vacuum pressure and osmosonication (V + US + OD) pretreatment prior to drying of garlic. It has been established that the use of vacuum technology in the pretreatment of food crops preceding drying improves mass transfer, enhances drying rate and preserves the physicochemical integrity of the finished product (Amiripour, Habibi-Najafi, Mohebbi, & Emadi, 2015; Feng et al., 2018). According to Feng et al. (2018), the application of pulsed vacuum osmotic dehydration enhances mass transfer rate, antioxidant and bioactive compounds, drying kinetics, drying rate, shortens the drying period and improves the energy efficiency of the drying process. Xule et al. (2019) reported that the vacuum osmotic dehydration pretreatment of scallion resulted in better rehydration, higher vitamin C retention, enhanced bactericidal effect, significantly reduced drying time and improved the quality of the finished product.

Quality assessment of botanicals (*i.e.* plant products) is usually a herculean task due to their multicomponent nature. Several studies have suggested the quantification of marker compounds as the basis for the quality control of these products. However, the possible synergistic roles of other components have led to calls for the holistic quality evaluation of botanicals. To this effect, chromatographic fingerprinting has become a recognized quality parameter by the WHO, FDA, SFDA and EMEA (Chen, Gao, & Sun, 2017). The use of this analytical technique reveals certain vital information about the chemical make-up of botanicals that hitherto remained hidden. For instance, it is capable of assessing batch-to-batch consistency, and determining the effect of various pretreament methods on the chemical integrity of the finished products (Wang, Zhang, Liu, Qian, & Fu, 2016). However, the information gotten using fingerprinting is only qualitative. It is therefore recommended that the advantages of these two quality assessment methods be leveraged upon for a better outcome. Hence, for quality evaluation of plant products, it is best to qualitatively assess their chemical make-up using fingerprinting as well as quantify their marker compound(s).

Herein, we aimed to: (1) compare the effects of US, US + OD and V + US + OD pretreatments on enzyme inactivation, antioxidant properties, total phenolic content, total flavonoid content, electrical energy consumption, rehydration ratio, and Fourier-transform infrared spectroscopy (FT-IR); (2) determine mathematical models that best describe the drying kinetics for each pretreatment method; (3) qualitatively assess the influence of the three pretreatment methods on the quality of the finished products using chromatographic fingerprinting and (4) quantitatively assess the marker compound, allicin, as a quality parameter to determine the effect of the various pretreatment methods. A schematic summary of the entire study is presented as Supplementary Fig. S1.

2. Material and methods

2.1. Fresh garlic samples

Fresh garlic samples were obtained from local producers at Binduri, Bawku East District in the Upper East Region of Ghana in June 2019 and transported to Nanjing, China after having obtained a phytosanitary certificate from the Plant Quarantine Division of the Ministry of Food and Agriculture, Accra, Ghana. The samples were further certified as true samples of *Allium sativum* by the lead author (a pharmacognosist). After thorough washing and cleaning, uniform slices (3–4 mm) were cut out (using SS-250, SEP Machinery Company Ltd., Guangzhou, China) and the initial moisture content determined according to the AOAC method (2000) to be 69.79 ± 0.81% (wet basis).

2.2. Chemicals and reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), sodium hydroxide (NaOH), Folin Ciocalteu reagent, sodium carbonate ($NaCO_3$), 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). Allicin reference compound (>95% purity, CAS No. 539–86-6) was bought from Bidepharm Co. Ltd (Shanghai, China). Purified water was obtained from Milli-Q water system (Millipore, USA).

2.3. Pretreatment methods

The ultrasonic equipment (Pulsed sweeping frequency ultrasonic equipment) manufactured by Wuxi Fanbo Biological Engineering Co., Ltd. China was employed for the sonication, osmosonication and vacuum-assisted osmosonication pretreatments. Water bath containing distilled water was operated at a fixed temperature (30 °C) for all processes. The 30% of $CaCl_2$ used for the pretreatment methods (US + OD and V + US + OD) in this study is an optimized concentration obtained after a series of preliminary studies in-house.

2.3.1. Control (untreated sample)

Fresh garlic slices (0.3 kg) were immersed in purified water in a beaker at ambient temperature for 40 min and then removed and cleaned with tissue paper.

2.3.2. US treatment

Sonication pretreatment was performed per the previously established protocol of Wang, Xu, Wei, and Zeng (2018) with slight modification. The fresh garlic slices (0.3 kg) were immersed in purified water in an Erlenmeyer flask. The Erlenmeyer flask containing the garlic slices was placed in the ultrasonic bath and sonication was performed at 40 kHz, for 40 min, using power of 600 W, at temperature of 30 °C and pulsating period of 10 s on-time and off-time. This process was replicated three times and the garlic samples were removed, drained and blotted with tissue paper.

2.3.3. US + OD pretreatment

The protocol of Osae et al. (2019) was employed with little adjustment with respect to the combined concurrent application of osmotic

dehydration and sonication. $CaCl_2$ of concentration 30% (w/v) was prepared in an Erlenmeyer flask and 0.3 kg of the garlic slices submerged in it at a ratio of 1:10 (sample: osmotic solution). This was then sonicated at a frequency of 40 kHz, for 40 min, using power of 600 W, at a temperature of 30 °C and pulsating period of 10 s on-time and 10 s off-time. This process was replicated three times and the garlic samples were removed, drained and blotted with tissue paper.

2.3.4. V + US + OD pretreatment

The method established by Feng et al. (2018) was followed. Fresh garlic slices (0.3 kg) were immersed in 30% (w/v) $CaCl_2$ 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 garlic slices were kept in the ultrasonic bath and operated using the following: frequency of 40 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. This process was replicated three times and the garlic samples were removed, drained and blotted with tissue paper.

2.4. Relative humidity convective drying

After the various pretreatment processes, the treated and untreated (control) garlic slices were dried in the relative humidity control dryer under the following conditions: temperature (60 °C), air velocity (2 m/s) and a constant relative humidity of 20%. Relative humidity convective dryer (RHCD) installed in the School of Food Science and Biological Engineering, Jiangsu University, China was employed in the current study. Fig. S2 is a schematic representation of the RHCD equipment. The RHCD equipment (lab-scale hot-air dryer equipped) works within a temperature range of 30–75 °C, relative humidity (10–40%), air velocity (2 ms^{-1}) and power of 1.2 kW. It consists of a water bath, weighing plate, loading tray (sieve), in-built electronic balance, circulating air fan, three thermocouples, electronic heating and axial flow fans, drying parameter switch, humidification and dehumidification systems as described in detail by Osae et al. (2019). Water bath (E5CN model, Omron, Tokyo, Japan) was used to regulate the temperature during the drying process. The sieve loader with an in-built electronic balance (Electronic Analytical Balance, FA 1104, China, Nanjing) was tared to zero before garlic samples were placed in it for further drying. This measures the weight of the garlic samples at 30 min interval at an accuracy of 0.1 g during the drying process. The electric heating system and axial (turbulent) flow fan which is part of the RHCD system blows and removes excess humid air from the drying chamber as well as control the relative humidity (RH) of the drying air within the drying compartment during the drying process. The humidification and dehumidification solenoid control system ensures that the RH is kept constant throughout the drying process. The relative humidity, temperature, drying time and drying stages are displayed on a touch screen. The RHCD also consists of 3 thermocouples with sensors (PT100, RTD Sensors Technology, China) that are located at various sections within the drying chamber and are usually inserted in the food material (*i.e.*, garlic slices) to determine the internal temperature of the sample (every 30 min) during the drying process.

2.5. Determination of total phenolic content (TPC) and total flavonoid content (TFC)

TPC and TFC of the dried pretreated garlic samples and the untreated were determined based on the reported method of Osae et al. (2019a). The results of TPC and TFC were respectively presented as mg gallic acid equivalents (GAE) per gram and mg catechin equivalents (CE) per gram of dry weight of the sample. For TPC determination, Folin-Ciocalteu reagent was diluted in distilled water at a ratio of 1:10 v/v. One milliliter of the extract was mixed with 5 mL of Folin reagent, then, 4 mL (75 g/L) 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 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). The methanol (80%) was used as the blank. The results obtained were expressed as mg of gallic acid equivalents (GAE) per gram g of dry weight of the sample.

With respect to TFC determination, the extracts (0.5 mL) were mixed with distilled water (2 mL) and 0.15 mL $NaNO_2$ solution (5%, v/v), for 6 min. Next, 0.15 mL of $AlCl_3$ solution (10%, v/v) was added to the mixture for 6 min and 2 mL of NaOH solution (4%, v/v) added to the mixture. Distilled water was then immediately added up to the final volume of 5 mL and the mixture allowed to stand for 15 min. The absorbance at 510 nm was determined by a spectrophotometer, Model TU-1810 (Purkinje Instrument Ltd., Beijing, China). The results were calculated as mg of catechin equivalents (CE) per gram of dry weight of the sample. The concentration range for catechin standard solutions was 6.25 to 100 mg/mL. The R^2 of the standard curve was 0.992.

2.6. Antioxidant activities

The potential source of antioxidant properties in the dried garlic slices pretreated by different methods (US, US + OD, V + US + OD) was determined based on the previous method of Osae et al. (2019) with slight modification. Assays of ABTS (2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), and DPPH (1,1-diphenyl-2-picrylhydrazyl) were measured using a spectrophotometer (Model UV-1600, Rayleigh Analytical Instrument, Beijing, China).

2.6.1. ABTs

The ABTS solution (7 mM) was added to potassium persulfate (2.45 mM) at a ratio of 1:1 and the resultant mixture kept in the dark at room temperature (25 °C) for 16 hr. 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 garlic 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.

2.6.2. DPPh

Briefly, 0.5 mL of the extracted garlic solution (supernatant) from each pretreated sample was added to 3 mL of methanol DPPH solution (60 mM) and vortexed. The mixture was kept for 30 min at room temperature in darkness. The absorbance (A_1) was read at 517 nm with a spectrophotometer, Model TU-1810 (Purkinje Instrument Ltd., Beijing, China) and the blank (A_0) was run with 3 mL of DPPH solution and 0.5 mL of methanol (80%).

The antioxidant activities were determined using equations (1) and (2) below:

$$ABTS\ radical\ scavenging(\%) = \frac{(A_0 - A_S)}{A_0} \times 100 \quad (1)$$

Where A_S represents the absorbance at 734 nm and A_0 as the blank

$$DPPH\ radical\ scavenging\ (\%) = \frac{(A_0 - A_1)}{A_0} \times 100 \quad (2)$$

where A_1 represents the DPPH absorbance at 517 nm with A_0 as the blank.

2.7. Rehydration ratio (RR)

The RR was conducted with reference to the previous established protocol of Osae et al. (2019) with slight adjustment. The RR of the dried garlic slices were measured by soaking in purified water at room

temperature (25 °C) for 6 h. The samples were then removed from the purified water, cleaned with tissue paper and weighed immediately at 1 h interval. After the stated time, the water was removed and samples were weighed. The RR was calculated using equation (3) below:

$$RR = \frac{Q_2}{Q_1} \tag{3}$$

where Q_1 is the weight of the garlic sample before RR and Q_2 is its weight after RR.

2.8. Fourier-transform infrared spectroscopy (FT-IR)

A mixture of lyophilized sample and potassium bromide was ground into powder, pressed into a thin sheet, then scanned (at 4000–400 cm^{-1}) at a resolution of 8 cm^{-1} using a Card-510A infrared spectrometer (Rayleigh, Beijing, China). The FT-IR spectra obtained were an average of 32 scans.

2.9. Specific energy consumption (SEC)

The *SEC* for each dehydration process was estimated using equation (4) below as previously reported by Shewale, Rajoriya, and Hebbar (2019): The electrical energy consumption during drying was estimated using electric energy meter (SZ-34, Havells's, India).

$$SEC(MJ/Kg) = \frac{CEV.3.6}{W_0} \tag{4}$$

Where
CEV = the Consumed energy value during drying process (kWh)
W_0 = Amount of moisture removed during drying (Kg)
SEC is specific energy consumption (MJ/kg)

2.10. Determination of polyphenol oxidase (PPO) enzyme activity

The method for the enzyme extraction and the assessment of PPO enzyme activity was performed with reference to the protocol of Osae et al. (2019b). Preparation of crude extract and measurement of enzyme activity are briefly outlined below.

2.10.1. Preparation of crude extract

Preparation of the enzyme extract solution was performed with reference to the method of Osae et al. (2019a) with slight modifications. The enzyme extract solution consisted of 0.2 M sodium phosphate buffer (pH 6.5), 1% (v/v) Triton X-100 and 2% (w/v) polyvinyl polypyrrolidone (PVPP). 2 g of the dried garlic powder was dissolved in 10 mL of the enzyme extract solution. The mixture was homogenized and kept for 5 min at 4 °C. The mixture was centrifuged at 7500 *g* for 30 min at 4 °C. The supernatant (PPO extract) was taken and the enzymatic activities were assessed immediately.

2.10.2. Measurement of enzyme activity

The PPO enzyme activity was measured with a spectrophotometer Model TU-1810 (Purkinje Instrument Ltd., Beijing, China). Polyphenol oxidase (PPO) assay mixture comprising 50 μL of the garlic enzyme extract and 0.1 mL of catechol (0.1 M) were added to 1.95 mL sodium phosphate buffer solution (pH 6.5) and vortexed for 10 s. The absorbance was estimated at 410 nm with the blank (same solution with no enzyme extract). 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 by the following equation:

$$Residual\ activity(\%) = A_t/A_0 \times 100 \tag{5}$$

Where A_t is the enzyme activity of the treated sample and A_0 is the enzyme activity of the control sample.

2.11. Mathematical modeling of drying kinetics

After the various pretreatment processes, the control (untreated) and the treated garlic slices (US, US + OD and V + US + OD) were dried at the same temperature of 60℃ and operating conditions in a relative humidity convective dryer as previously described by Osae et al. (2019a). Mathematical models (Table S1) were then assessed to determine the appropriate model that best fitted the drying curves of the garlic slices for each pretreatment method. Drying rate and moisture ratio of garlic slices for the period of drying were calculated using equations (5) and (6) below as previously reported (Osae et al., 2019a):

$$MR = \frac{M_t - M_e}{M_0 - M_e} \tag{6}$$

where M_o is the initial moisture content, M_t is the moisture content at the drying time t, M_e is the equilibrium moisture content and MR is the moisture ratio. Me is the equilibrium moisture content and usually termed as Me (equilibrium moisture content) when the dried sample attains a constant moisture content.

$$DR = \frac{M_{t1} - M_{t2}}{t_2 - t_1} \tag{6}$$

where M_{t1} and M_{t2} are the moisture contents of garlic samples at varied drying times (t_1 and t_2) expressed on a dry weight basis. And t_1 and t_2 are the drying times in minutes at the various time- points during the drying process.

To evaluate the quality fitness of the experimental results, reduced chi-square (χ^2), the regression coefficient (R^2) and reduced sum of square (RMSE) were used. Lower RMSE and Chi-square ($\chi 2$) values with higher coefficient (R^2) values were used to measure the appropriateness of the model to describe the drying curves of the untreated, US-, US + OD - and V + US + OD -pretreated samples. These parameters were calculated using equations (7–9) below (Dai et al., 2015; Wang et al., 2018):

$$\chi^2 = \frac{\sum_{i=1}^{N}\left(MR_{exp,i} - MR_{pre,i}\right)^2}{N - z} \tag{7}$$

$$RMSE = \sum_{i=1}^{N}\left(MR_{pre,i} - MR_{exp,i}\right)^2 \tag{8}$$

$$R^2 = \frac{\sum_{i=1}^{N}\left(MR_{pre,i} - MR_{exp,i}\right)^2}{\sum_{i=1}^{N}\left(\overline{MR_{pre}} - MR_{exp,i}\right)^2} \tag{9}$$

where *z* stands for number of constants; *N,* the number of experiences; $MR_{exp,i}$, experimental dimensionless moisture ratio and $MR_{pre,i}$ is the computed dimensionless moisture ratio.

2.12. Content of allicin determination

2.12.1. Sample preparation

A 200 mg quantity of each powdered sample was measured into a 2 mL Eppendorf tube, and 500 μL of methanol added. The samples were vortex-mixed and subjected to ultrasound-assisted extraction using ultrasonic water bath (KQ3200DE, Kunshan, China) at 25 °C, 100 Hz for 45 min. Thereafter, they were centrifuged at 13000 rpm for 10 min at 4 °C using Eppendorf 5430R centrifuge and the supernatant filtered through 0.22 μm sintered glass filter. The filtrate was then used for HPLC analysis. Standard solutions of allicin of concentrations, 0.625, 1.25, 2.5, 5, 10 mg/mL were prepared from a stock solution of 20 mg/mL via serial dilution using methanol as the solvent. All samples were stored at −80 °C until analysis.

2.12.2. HPLC analysis

The content of allicin in the pretreated samples was determined by HPLC analysis. This analysis was achieved using Agilent 1260 HPLC set up with a variable wavelength detector (VWD) and auto sampling compartment. Separations were effected by isocratic elution of 0.1% aq. formic acid and acetonitrile in the ratio of 50:50 using an Inertsil ODS-SP column (5 μm, 4.6 × 250 mm, GL Sciences Inc., Japan). With an injection volume of 10 μL and flow rate of 1.0 mL/min, the analysis was done at a column temperature of 30 °C. Separated components were detected at 305 nm via ChemStation software and validated based on the ICH guidelines for linearity, accuracy, precision, and limits of detection and quantification. The peak corresponding to allicin in each sample was identified based on its retention time relative to that of the reference compound under same conditions and confirmed via UHPLC-Q/TOF-MS analysis. Same mobile phase composition was used but performed on a Zorbax Eclipse Plus C_{18} (2.1 × 100 mm, 1.8 μm) column using the Agilent 1290 LC system coupled to an Agilent 6545 quadrupole time-of-flight spectrometric (Q/TOF-MS) detector at a flow rate of 0.4 mL/min using an injection volume of 1 μL. The operating parameters (mass spectrometry) were as follows: drying gas temperature, 350 °C; drying gas flow rate, 12 L/min; nebulizer gas, 40 psi; fragmental voltage, 120 V and capillary voltage, 3500 V. This confirmatory analysis was performed in the positive ion mode.

2.13. Chromatographic fingerprint (similarity) analysis

To assess the similarity or dissimilarity among the pretreated samples, the following method was used. Chromatographic separations were carried out using same HPLC equipment and as earlier indicated for allicin content analysis (*i.e.* Agilent 1260 HPLC). The same mobile phase of 0.1% aq. formic acid (mobile phase **A**) and acetonitrile (mobile phase **B**) was used. However, the following gradient elution was used: 0–10 min, 5% **B**; 10–18 min, 5–15% **B**; 18–25 min, 15–45% **B**; 25–35 min, 45–75% **B**; 35–40 min, 75–100% **B**; 40–45 min, 100–5% **B** with 10 min of post run. Detection of all eluted components was done at 254 nm. This method captured as many chemical components as possible. The representative chromatogram obtained for each pretreated sample was converted to the AIA format and analyzed using the *Chromatographic Similarity Evaluation software* (2004A). These chromatograms were overlaid and a reference chromatogram generated. Similarity between each pretreated sample to reference chromatogram was determined as cosine ratios.

2.14. Statistical analysis

Data obtained from triplicate determinations were given as mean ± standard deviation values. Comparison of the means of the various determinations was done using the Tukey test (Minitab Inc., Pennsylvania, USA). P-values < 0.05, were considered statistically significant. Drying kinetics data was analyzed using OriginPro software 2018. Graphical analyses and depictions of other quality parameters were achieved using GraphPad Prism 6 (Inc. San Jose, CA, USA).

3. Result and discussion

3.1. TPC and TFC of dried garlic slices under various pretreatment techniques.

The results of the bioactive compounds (TPC and TFC) of dried garlic slices pretreated by the various methods (V + US + OD, US + OD, US and untreated) are summarized in Table 1. Therein, the TPC of dried garlic slices are 68.07, 57.34, 43.67, and 24.29 mg GAE/gdw for the V + US + OD -, US + OD -, US-pretreated samples and the untreated sample respectively. The TFC of garlic slices pretreated by V + US + OD, US + OD, US and untreated sample are 54.17, 44.76, 37.98 and 16.68 mg CE/gdw respectively. The bioactive compounds (TPC and TFC) of V + US + OD-pretreated samples were significantly ($P < 0.05$) higher than the US + OD and US-pretreated samples. This difference could be attributed to the modus operandi of this technique. The combined additive influence of the acoustic cavitation of US and vacuum pressure as well as OD pressure gradient, weakened and disrupted the cell structure leading to the creation of several micro pores within the surface of the garlic tissue (Osae et al., 2019b). This aided the diffusion of moisture, enhanced the drying rate, reduced the drying time and retained greater percentage of the bioactive compounds than the US + OD- and US-pretreated slices. This outcome is in agreement with the previous findings of Feng et al. (2018), who reported that the propagation of V + US + OD through a liquid medium produces a variety of effects such as acoustic cavitation, sponge effect, acoustic streaming or micro-streaming and microscopic channels as well as reduction in pressure which causes an expansion of the air in the intercellular spaces and pores of the sample via hydrodynamic mechanisms due to the pressure difference. This greatly improved the mass transfer, lessened the drying time and preserved the quality of dehydrated Chinese garlic slices (Feng et al., 2018).

Table 1
Antioxidant activities, TPC and TFC of garlic slices under different pretreatment conditions.

Pretreatments	Antioxidant activities		Bioactive compounds	
	ABTS (%)	DPPH (%)	TPC (mg GAE/gdw)	TFC (mg CE/gdw)
Control	20.23 ± 0.30^{d}	34.53 ± 1.41^{d}	24.29 ± 0.70^{d}	16.08 ± 4.14^{d}
US	36.43 ± 2.7^{c}	51.97 ± 1.46^{c}	43.67 ± 5.84^{c}	37.98 ± 5.84^{c}
US + OD	54.17 ± 0.40^{b}	70.77 ± 6.83^{b}	57.34 ± 0.68^{b}	44.76 ± 0.64^{b}
V + US + OD	69.67 ± 4.73^{a}	77.83 ± 3.24^{a}	68.07 ± 2.27^{a}	54.17 ± 0.40^{a}

Liu et al. (2013) who studied the impact of US + OD pretreatment of pomegranate peel reported that, the influence of OS increased tissues softening of the carrot sample, enhanced higher mass transfer and retained the bioactive compounds. In agreement with these findings, Osae, Zhou, Alolga et al. (2019) reported that US + OD pretreatment preceding drying of ginger led to a higher retention of bioactive compounds. Furthermore, Ren et al. (2018) and Zhang et al. (2017) established that the application of US pretreatment prior to drying of onion and daylilies led to a greater retention of TPC and TFC.

3.2. Antioxidant activities of garlic slices under different pretreatment conditions.

The influence of different pretreatments (V + US + OD, US + OD, US and untreated) on the antioxidant capacities of the dried garlic slices are presented in Table 1. ABTS and DPPH assays were employed to measure the potential source of antioxidation in the pretreated and untreated dried garlic samples. The ABTS antioxidant abilities of V + US + OD, US + OD, US and the untreated dried garlic slices are respectively 69.67, 54.17, 36.43 and 20.23%. The outcome of the DPPH assay were 77.83, 70.77, 51.97 and 34.53% for V + US + OD, US + OD, US and the untreated sample respectively. The results of both assays followed a similar trend: V + US + OD -pretreated garlic slices > US + OD-pretreated garlic slices > US-pretreated garlic slices > untreated garlic slices.

This consistent and statistically significant ($P < 0.05$) higher antioxidant activities of the V + US + OD -pretreated slices could again be attributed to the modus operandi of the method as earlier explained under TPC and TFC assay. A similar observation was made by Xule et al. (2019). Our results further established an association between antioxidation and TPC, since higher TPC is usually attributed to an increase in antioxidant activities. Hence, the results of antioxidation of the treated garlic slices are consistent with that of TPC determination.

The highest DPPH antioxidant activity and TPC recorded for V + US

+ OD-pretreated samples may be due to the shorter drying time of this pretreatment technique, as the less drying time preserved a greater percentage of the antioxidant properties and phenolic compounds compared to the US- and US + OD-pretreatments. Also, we found a link between the TPC and the antioxidant activity, which suggests that the increased antioxidant activities could at least partly be ascribed its high TPC. According to Osae et al. (2020), the application of non-thermal pretreatment techniques such as US + OD and V + US + OD hasten the formation of free radicals and enhance the polymerization of phenolic compounds and antioxidant properties. This is in agreement with the report of Hamedi, Mohebbi, Shahidi, and Azarpazhooh (2018).

3.3. Specific energy consumption

Fig. 1A shows the specific energy consumption of pretreated and untreated garlic samples dried under RHCD. The consumed energy value (kWh) for the V + US + OD, US + OD, US, and the control (untreated) were 4.8, 5.4, 6.6, and 11.4 kWh/Kg respectively. As depicted in Fig. 1A, specific energy consumption levels for the V + US + OD, US + OD, US, and the control (untreated) were 19.20, 23.14, 27.29, and 47.72 MJ/Kg respectively. This represents a percentage decrease in energy consumption of 36.21, 48.49 and 57.19% for the V + US + OD, US + OD and US pretreatment methods respectively. This therefore shows that V + US + OD pretreatment is more energy efficient than the other methods. The reduced energy consumption of V + US + OD pretreatment lends credence to the enhanced mass transfer, higher drying rate and shorter drying time of this technique.

Mierzwa and Kowalski (2016) reported that the application of US + OD pretreatment preceding convective drying of carrot slices reduced the energy consumption compared to the untreated samples. They further explained that the influence of US + OD pretreatment significantly enhanced dewatering (greater water loss) efficiency and reduced the total energy consumption leading to the reduction in the total dehydration time. Previous works of Kroehnke, Musielak, and Boratynska (2014), observed that the use of US-assisted convective drying of potato decreased the energy consumption and improved the quality of the dried potato slices.

3.4. Rehydration ratio (RR)

The elimination water from the cell structure of food samples during drying results in alterations in the physical characteristics of the dehydrated product. It is defined as the ability of dehydrated sample to maintain its unique state after absorption of water (Tüfekçi & Özkal, 2017). Fig. 1B depicts the RR of dried garlic slices pretreated by V + US + OD, US + OD, US and the untreated (control).The dehydrated garlic slices treated with V + US + OD showed higher RR compared to the US + OD and US. This may be explained by the creation of several micro pores on the surface of the garlic samples due to the V + US + OD treatment which improved the water absorption during the RR process. The maximum RR ability was consistent with the establishment of a higher porous microstructure. The higher RR of US + OD -treated garlic slices relative to the US-treated samples may be the result of the synergistic effect of sonication cavitation and osmotic pressure which damaged the cell structure by the formation of micro channels and promoted the movement of water into the dried garlic slices. Osae et al. (2019b) and Çağlayan and Barutçu Mazı (2018) established a similar observation for dried ginger and pumpkin slices.

The US-pretreated dehydrated garlic slices attained higher RR relative to the untreated samples. This could be attributed to the action of ultrasonic cavitation on the exterior tissue of the garlic samples preceding drying which led to the formation of pores and enhanced movement of water into the dried sample. This finding is in tandem with the reports of Bozkir et al., (2019) and Wang et al., (2018) for garlic and carrot respectively.

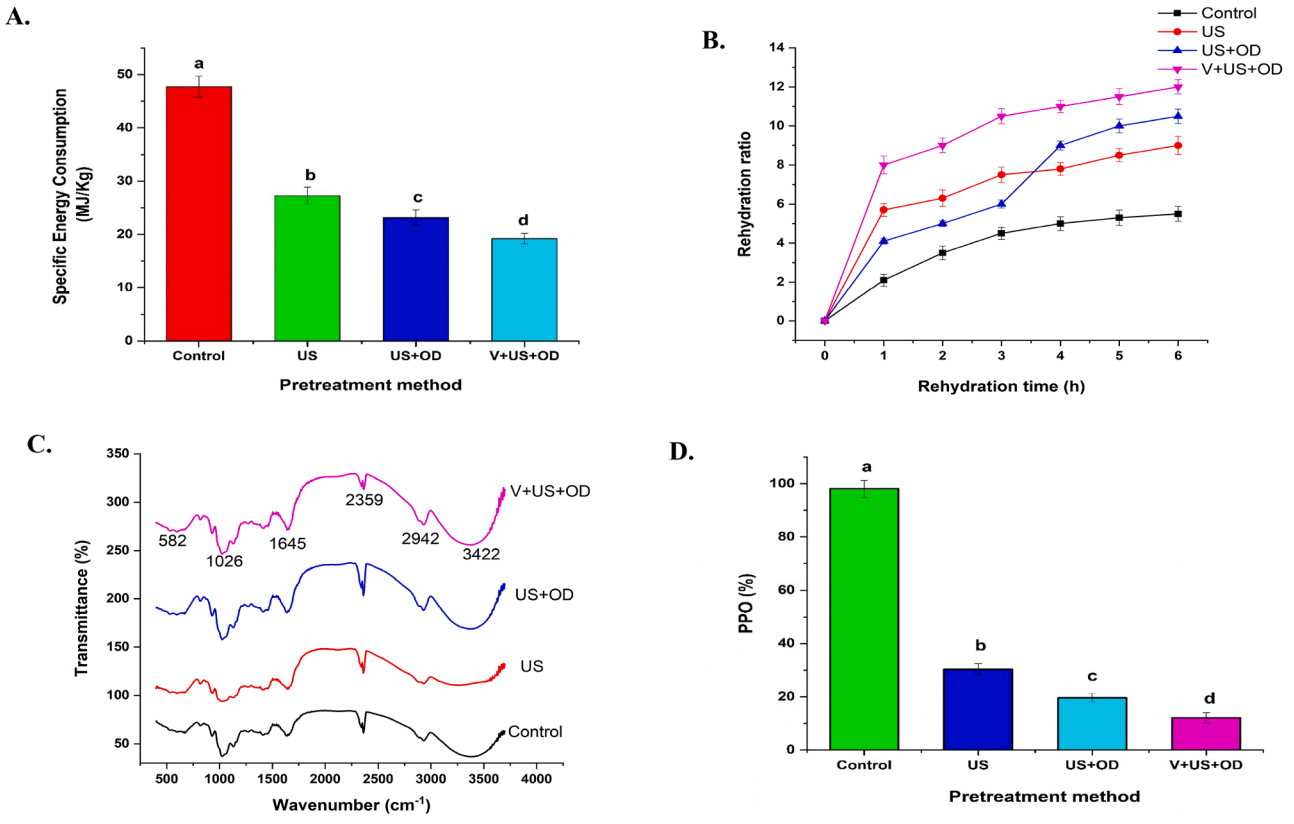


Fig. 1. Effect of different pretreatments on (**A**) Energy consumption (**B**) Rehydration ratio (**C**) FTIR spectra and (**D**) residual enzyme activity of PPO. The letters on data bars (a, b, c, d) symbolize a significant difference ($P < 0.05$).

3.5. Fourier transform infrared (FTIR) spectroscopic analysis

FTIR spectroscopic study was conducted to determine the key structural changes which might have taken place due to the application of the various pretreatment methods. The outcome of the analysis has been shown in Fig. 1C.

All samples exhibited common characteristic peaks at 3422, 2942, 2359, 1645, 1026 and 582 cm^{-1}. The strong, broad peak at 3422 cm^{-1} is characteristic of O—H stretching (Liming Zhang, Guan, Zhang, Dai, & Hao, 2018). This peak is more pronounced in the V + US + OD-pretreated sample followed by US + OD-pretreated sample compared to the control (untreated). For the US-pretreated sample, the shape of this peak is quite uncharacteristic compared to the other samples. This could be due to either the weakening of the O—H bond or the gradual loss of this bond through the pretreatment process. Also the peak at 2942 cm^{-1}, which is representative of C—H stretching vibration, is less pronounced in the US-pretreated sample (Liming Zhang, Guan, Zhang, Dai, & Hao, 2018). A phenomenon similar to that of the O—H bond could have occurred in this case leading to either the weakening of this bond or its gradual loss. The other peaks were more pronounced in the V + US + OD- and US + OD-pretreated samples compared to the US-pretreated sample and the control. The signal at 1645 cm^{-1} represents the C═C stretching vibrations of the allyl group while that at 1026 cm^{-1} is characteristic of S═O (sulfinyl group) vibrations (Liming Zhang, Guan, Zhang, Dai, & Hao, 2018)

These findings suggest V + US + OD and US + OD as the best and second best pretreatment methods respectively in terms of maintaining the structural bond integrity of the garlic samples. Our findings also point out the differences between these two pretreatment methods and US.

3.6. Influence of pretreatment techniques on the drying kinetics of garlic slices

A graphical presentation of the drying kinetics (moisture ratio against drying time) of the garlic slices pretreated by different methods (US, US + OD, V + US + OD) as well as the untreated (control) sample is depicted in Fig. 2A. The initial moisture content (MC) of the fresh garlic slices was 69.79% and the assessment of the MC in the course of the drying process was done at 30 min interval until constant MC was achieved. As can be noticed in Fig. 2A, pretreatment improved drying compared to the untreated (control) sample. The respective drying duration for the V + US + OD-, US + OD -, US-pretreated samples and the untreated garlic slices was 4 h (280 min), 5 h (300 min), 5.5 h (330 min) and 9.5 h (570 min). Our results clearly established that the different pretreatment techniques employed lessened the drying duration by 57.90%, 52.63% and 49.12% for V + US + OD, US + OD and US respectively. Wang et al., (2018) reported that the application of US pretreatment for carrot slices prior to drying led to a 25% decrease in the drying time. They further stated that the influence of US resulted in the

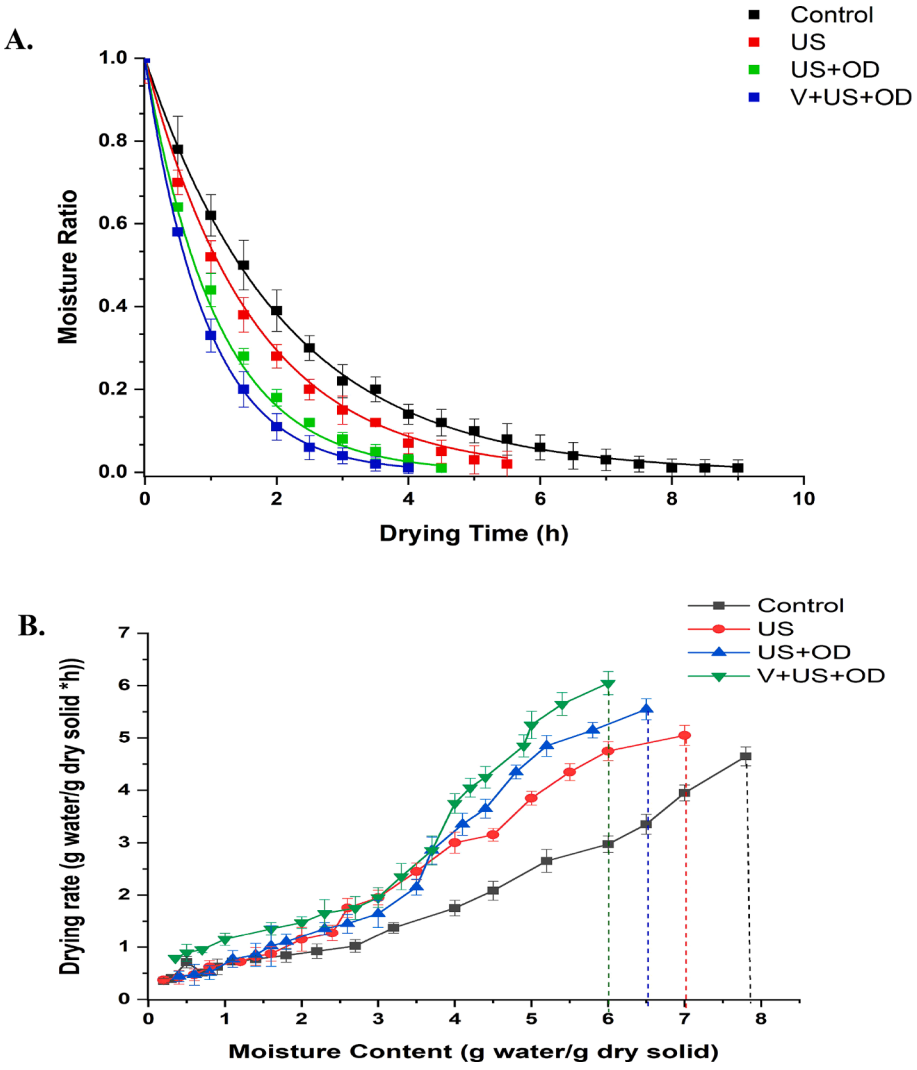


Fig. 2. Effect of different pretreatments on the moisture kinetics (**2A**) and drying rate (**2B**) of garlic samples.

disruption of the cell structure and enhanced moisture diffusion during the dehydration process. Osae et al. (2019a) pretreated ginger slices using US + OD prior to drying and established a reduction in the drying time of about 50% compared to untreated ginger slices. The reduced drying time by the US + OD pretreatment could be the result of the formation of several micro pores inside the cell structure of the garlic slices ultimately resulting in increased moisture migration and enhanced drying time. The best drying time of the V + US + OD -pretreated could be credited to the enhanced mass transfer due to the creation of several micro pores and the disruption of the cells structure due to the combined effect of US and vacuum-assisted OD. This outcome is in tandem with the work of Cheng et al [18].

As shown in Fig. 2B, the various pretreatment techniques (US, US + OD and V + US + OD) advanced the drying rate (DR) of garlic slices compared to the untreated samples. There was a gradual increase in DR throughout the whole drying period with the V + US + OD -pretreated sample recording higher values than the US + OD - and US-pretreated samples. The reasons for the differential drying duration (*i.e.* moisture content kinetics) could also be assigned this instance is the trend and phenomenon is same. Previous studies have also reported similar findings for some of these pretreatment methods. For instance, Zhang et al. (2017) who employed US pretreatment preceding the dehydration of daylilies reported an enhanced the DR compared to the untreated samples. Also, Dehghannya, Gorbani, and Ghanbarzadeh (2015) showed that US + OD pretreatment of Mirabelle plum improved its DR.

3.7. Modeling of drying kinetics of pretreated garlic slices

To evaluate the models that best fitted the drying curves of the garlic slices pretreated by various techniques (US, US + OD, V + US + OD and the control), five mathematical models including Page, Henderson and Pabis, Two term exponential, Lewis and Diffusion Approach models (Table 2) were the models that were used. To predict the appropriate model that best describes the experimental data, the following statistical parameters were employed; coefficients of determination (R^2), root means square error (RMSE) and Chi square (χ^2). The best model was the one with the highest R^2 and lowest RMSE and χ^2 values. As shown in Table 2, the Henderson and Pabis model recorded the highest R^2 and lowest RMSE and χ^2 values for both the pretreated and untreated garlic sample making it the best model compared to the rest. Other researchers such as Torki-Harchegani et al., (2016) and Elhussein and Şahin (2018) found this model to best describe their experimental data in the drying of Peppermint and olive leaves respectively.

Table 2
Modeling of drying kinetics of garlic slices under different pretreatment methods.

Pretreatments	Model name	R^2	$\chi^2 \times 10^{-5}$	RMSE
Control	Page	0.9992	6.8967	0.00811
	Henderson and Pabis	**0.9995**	**6.584**	**0.0083**
	Two term exponential	0.9991	6.9443	0.008617
	Lewis	0.9992	7.51	0.00833
	Diffusion Approach	0.9990	7.62	0.008578
US	Page	0.9954	48.5029	0.02209
	Henderson and Pabis	**0.9975**	**44.4501**	**0.01776**
	Two term exponential	0.9961	48.7758	0.0183
	Lewis	0.9955	56.9401	0.02108
	Diffusion Approach	0.996	59.4901	0.01829
US + OD	Page	0.9970	37.2238	0.0145
	Henderson and Pabis	**0.9994**	**32.8814**	**0.0138**
	Two term exponential	0.9972	37.84	0.0149
	Lewis	0.9962	39.0013	0.01929
	Diffusion Approach	0.9971	88	0.01518
V + US + OD	Page	0.9984	28.52	0.01394
	Henderson and Pabis	**0.9997**	**19.4308**	**0.01016**
	Two term exponential	0.9977	35.4504	0.01295
	Lewis	0.9966	38.3528	0.01883
	Diffusion Approach	0.9985	24.1784	0.01024

3.8. Effect of pretreatment methods on PPO inactivation

Fig. 1D depicts the influence of V + US + OD, US + OD and US pretreatment on the residual enzyme inactivation of PPO of dehydrated garlic slices. In this regard, a statistically significant ($P < 0.05$) difference was found among the pretreatment methods with percentage PPO residual activity (RA) of 13.21%, 19.64% and 30.16% for V + US + OD, US + OD and US respectively. This shows that V + US + OD pretreatment was the best in terms of inactivating the PPO enzyme compared to US + OD and US. The minimum enzyme RA obtained for the V + US + OD -pretreated sample could be ascribed to the effects of vacuum pressure, osmotic pressure and ultrasonic cavitation waves which together created unfavorable conditions for enzymatic activity. From Fig. 1D, it is evident that US + OD pretreatment was better than US at inactivating the PPO enzymes. The probable underlying reason could be impact of cavitation by ultrasound and osmotic pressure resulting in degradation of enzyme structure and activity. This assertion is in line with the report of Osae et al. (2019) and Cao, Cai, Wang, and Zheng (2018) for PPO enzyme inactivation of ginger and bayberry. Finally, the PPO enzyme inactivation activity in the US-pretreated sample was better than the control (untreated) due to the mechanical and chemical effects of ultrasonic cavitation on the enzyme system leading to their breakdown.

3.9. Effect of various pretreatments on the non-volatile chemical integrity of garlic

3.9.1. Allicin content

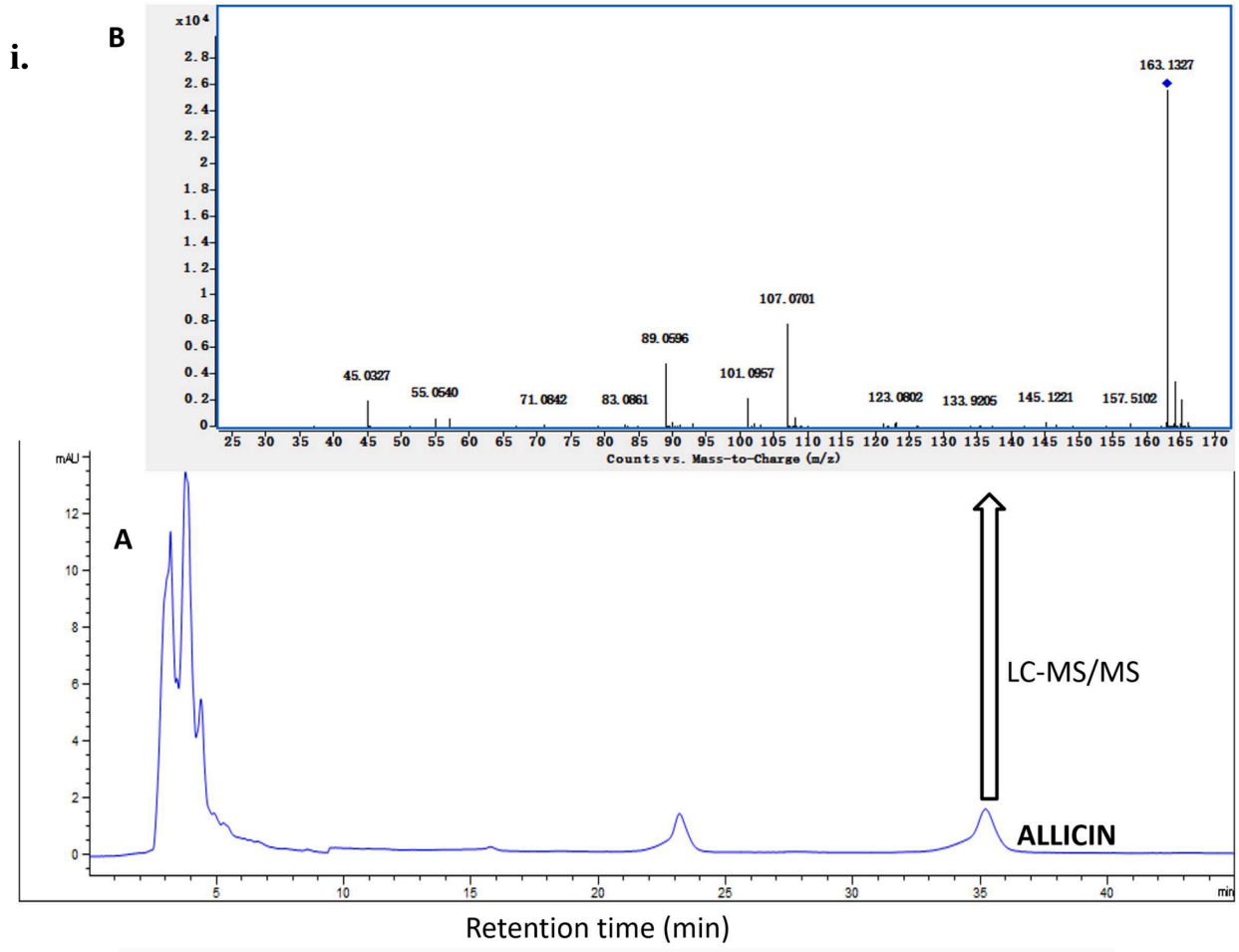
The content of allicin in the finished products was determined as a basis to evaluate the effects of the various pretreatment methods on their quality. Using HPLC-UV analysis, a simple method was developed and duly validated per the ICH recommendations. As shown in Fig. 3i.(AB), the retention time of allicin was 35.1 min and its identity was confirmed by LC-MS/MS analysis by comparison of its fragmentation pattern to that of the reference compound. The method was linear within the concentration range used (0.625–10 mg/mL) producing a line of equation, $y = 217.24x + 18.911$ ($R^2 = 0.998$). The method was also accurate (recovery range, 96.9–99.8%) and precise with relative standard deviation values for intra- and inter-day precision within acceptable limits. The LOD and LOQ values were respectively 0.8 μg/mL and 2.5 μg/mL. Details of the outcome of the validation procedure is tabulated as Supplementary Table S2.

As depicted in Fig. 3**(ii)**, the content of allicin was as follows: 8.292 ± 0.121, 3.908 ± 0.397, 2.901 ± 0.882, and 0.575 ± 0.225 for the V + US + OD-, US + OD-, US-pretreated samples and control (untreated sample) respectively. These results confirm the superiority of V + US + OD pretreatment over US + OD, and US. The modus operandi of this technique as earlier explained maintains the chemical integrity of the garlic slices as evidenced by the high content of allicin (a known quality marker) after pretreatment. Per the recommendations of the British Pharmacopoeia, only the V + US + OD-pretreated sample meets the criterion of a minimum allicin content of 4.5 mg/g making this the recommended pretreatment method for garlic (Khar, Banerjee, Jadhav, & Lawande, 2011).

3.9.2. Chromatographic fingerprint

To get a holistic view of the impact of the pretreatment methods on the chemical composition of the garlic samples, we compared their chromatographic fingerprints using the similarity software (Fig. 4). Using a reference chromatogram generated from all samples, its similarities with the samples were determined as cosine ratios. The nearer the values to 1, the greater the similarity to the reference chromatogram.

As can be observed from Supplementary Table S3, the trend of similarity was: V + US + OD > US + OD > CTL > US. These results suggest differential levels of all compounds (not just allicin) in the various pretreated samples with that of the US-pretreated sample being



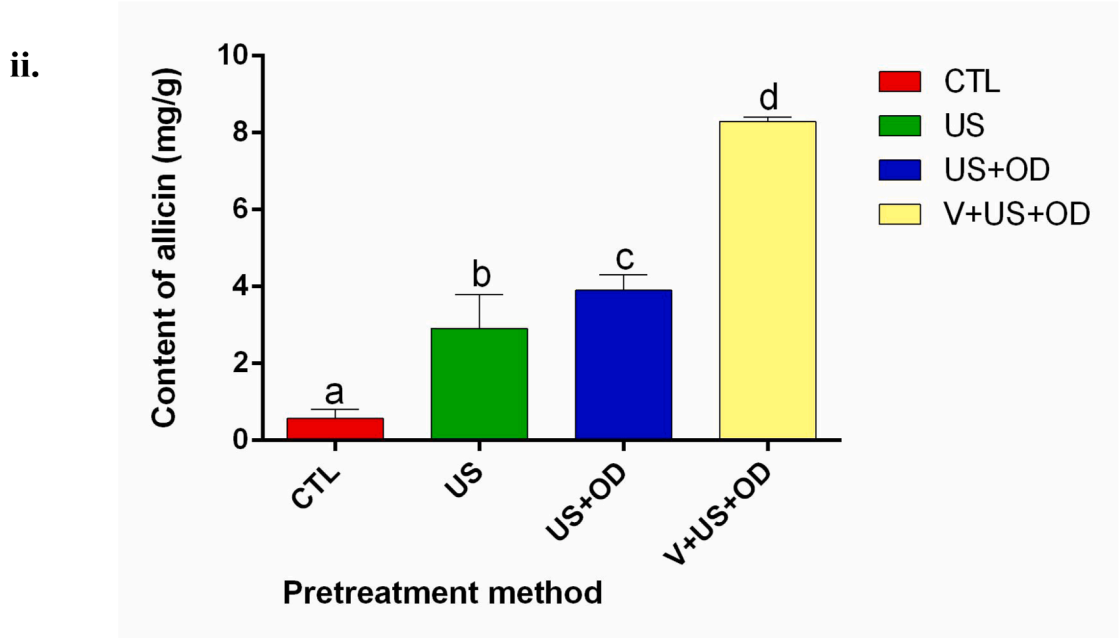


Fig. 3. (i) Typical HPLC-UV chromatogram of garlic samples showing the position of allicin (**A**) and its confirmatory LC-MS/MS spectrum (**B**). **(ii)** Graphical depiction of allicin content in various pretreated samples of garlic and the control (untreated) sample. The letters on data bars (a, b, c, d) symbolize a significant difference ($P < 0.05$).

quite distinct. Our findings also lend support to that of the FT-IR where differences in the chemical make-up of the finished products were detected. However, chromatographic fingerprinting is qualitative but when combined with quantitative analysis provides credible and valid information. Hence the outcome of the chromatographic fingerprinting and allicin content taken together, it can be concluded that VOS pretreatment prior to drying is the best method among the three.

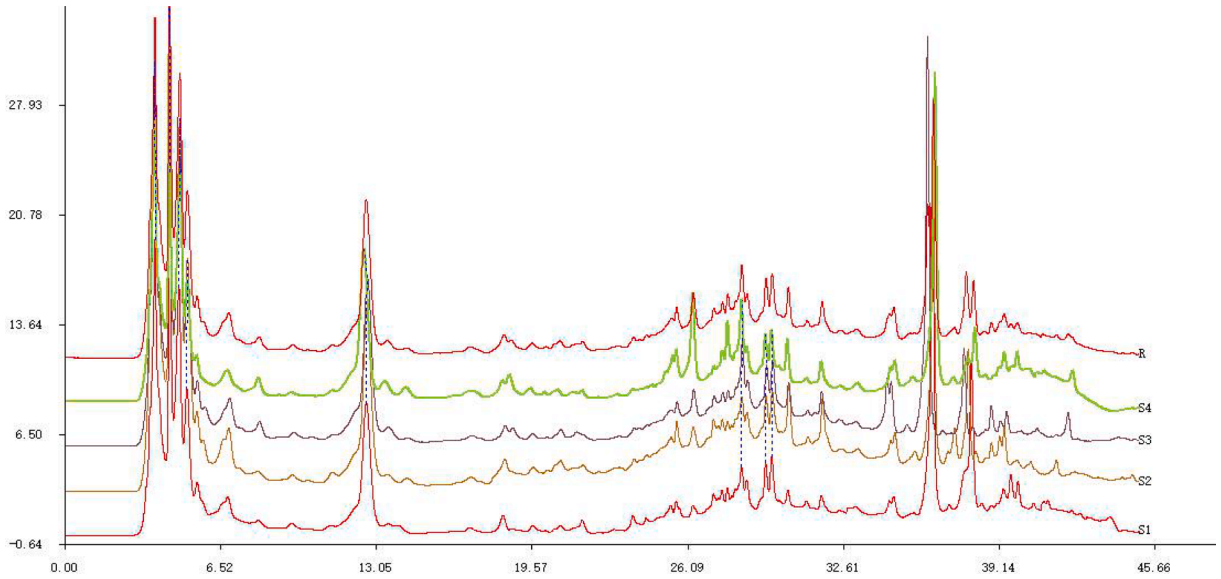


Fig. 4. Pictorial depiction of chromatographic fingerprinting of the garlic samples. S1 = CTL; S2 = US; S3 = US + OD; S4 = V + US + OD; R = reference chromatogram.

4. Conclusions

The outcome of all determinations taken together, it can be concluded generally that V + US + OD pretreatment is the best method to be employed prior to the drying of garlic compared to US + OD and US. Specific conclusions however include (1).V + US + OD pretreatment led to enhanced activities in respect of antioxidant properties, TPC, TFC and PPO enzyme inactivation of the finished product. (2). V + US + OD pretreatment gave higher RR, resulted in the shortest drying time and was more energy efficient. (3). V + US + OD pretreatment maintained the chemical integrity of the finished product as evidenced from the results of the FT-IR determination, chromatographic fingerprinting and allicin content determination. (4). V + US + OD-pretreated samples recorded the highest content of allicin and met the specification of the British Pharmacopoeia for garlic products meant for consumption (*i.e.* minimum content of 4.5 mg/g). In future studies, a slightly different methodological approach including more controls would be adopted. Finally, fresh garlic samples from other parts of the world would be compared in order to confirm or otherwise our current findings.

CRediT authorship contribution statement

Raphael N. Alolga: Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Writing - original draft. **Richard Osae:** Conceptualization, Investigation, Methodology, Software, Validation, Writing - original draft. **Gloria Essilfie:** Conceptualization, Supervision, Writing - review & editing. **Firibu Kwasi Saalia:** Methodology, Resources, Supervision, Writing - review & editing. **Selorm Akaba:** Methodology, Resources, Software, Writing - review & editing. **Fadzai Chikari:** Investigation, Software.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.128535.

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