Nondestructive monitoring, kinetics and antimicrobial properties of ultrasound technology applied for surface decontamination of bacterial foodborne pathogen in pork

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ARTICLE INFO
Keywords:
Ultrasound
Bacterial foodborne pathogens
Electronic nose
Hyperspectral imaging
Kinetics
Chemometrics

ABSTRACT
In this study, electronic nose (E-nose) and Hyperspectral Imaging (HSI) was employed for nondestructive monitoring of ultrasound efficiency (20KHZ) in the inactivation of Salmonella Typhimurium, and Escherichia coli in inoculated pork samples treated for 10, 20 and 30 min. Weibull, and Log-linear model fitted well (R² ≥0.9) for both Salmonella Typhimurium, and Escherichia coli inactivation kinetics. The study also revealed that ultrasound has antimicrobial effects on the pathogens. For qualitative analysis, unsupervised (PCA) and supervised (LDA) chemometric algorithms were applied. PCA was used for successful sample clustering and LDA approach was used to construct statistical models for the classification of ultrasound treated and untreated samples. LDA showed classification accuracies of 99.26%, 99.63%, 99.70%, 99.43% for E-nose-S. Typhimurium, E-nose-E. coli, HSI-S. Typhimurium and HSI-E. coli respectively. PLSR quantitative models showed robust models for S. Typhimurium-(E-nose-R² = 0.9375, RMSEP = 0.2107 log CFU/g and RPD = 9.7240) and (HSI Rp² = 0.9687 RMSEP = 0.1985 log CFU/g and RPD = 10.3217) and E. coli-(E-nose-Rp² = 0.9531, RMSEP = 0.2014 log CFU/g and RPD = 10.1731).

This novel study shows the overall effectiveness of applying E-nose and HSI for in-situ and nondestructive detection, discrimination and quantification of bacterial foodborne pathogens during the application of food processing technologies like ultrasound for pathogen inactivation.

1. Introduction

The incidence of foodborne outbreaks caused by eating meat and poultry products remains a substantial public health concern and poses serious questions regarding meat health among consumers. Therefore, the production of meat and meat products does not only concern the assembly lines in slaughter meat plants but the prevention of microbial contaminants and the destruction of spoilage and microbial foodborne pathogens. Pathogen decontamination in the industry has traditionally been achieved through some chemical and physical methods such as chemical dehairing, carcass washing and the use of substances with antimicrobial properties. Increased awareness among consumers of health risks associated with food chemicals and antimicrobials has led to the development of alternate and advanced food preservation techniques such as ultrasound (US) [1].

Future food safety developments across the world and in relation to the meat industry call for appropriate interventions across the entire meat production line. Ultrasound is a “green”, non-thermal and non-destructive technology which works on the mechanism of creating acoustic cavitation in a liquid media while creating vibrational frequencies believed to be the principal mechanism responsible for observed changes in exposed materials [2]. As non-chemical application technology, it has gained broader application in the meat industry with recent studies focusing on a range of improving food quality and safety characteristics such as improving tenderness, bacteria and enzyme inactivation.

The application of ultrasound for bacteria inactivation has been widely studied [3–5]. Furthermore, the use of ultrasound in meat...
processing increases meat quality standards, such as tenderness. In general, high strength and high-intensity (> 1 W cm\(^{-2}\)) ultrasound are used in food processing at frequencies between 20 and 500 kHz [6].

However, the traditional microbiological methods for monitoring the effect of ultrasound (US) treatment on meat products which includes culture methods are invasive, labour intensive and time-consuming. These methods are becoming obsolete in the face of technological advancement in autonomous, machine-lead food processing lines which rely on speed and non-contact applications. To scale up and commercialise ultrasound application in the food (meat) industry, monitoring regimes that are complementary with the latest food processing techniques must be applied to rapidly measure the effects of ultrasound application nondestructively.

HSI is an imaging spectroscopic technique that integrates computer vision (imaging) and vibrational spectroscopy such that both spatial and spectral information variables for each pixel in the image are collected simultaneously. The fundamental HSI principle for the identification of microorganisms is based the premise that bacteria metabolites provide characteristic fingerprints signaling infection by microorganisms in food samples [7].

E-nose on the other hand is an artificial olfaction system capable of sensing volatile organic compounds and odour expression. The feasibility of microbial volatile organic compound sensing for rapid prediction and discrimination of bacterial foodborne pathogens has been extensively studied.

In recent years, the development of nondestructive methods like hyperspectral imaging (HSI) [8,9] and the electronic nose (E-nose) [10,11] in food industry applications for the detection of bacterial foodborne pathogens is growing with increased success. All of these studies indicate that HSI and E-nose are ideal for online monitoring of pathogen deactivation programs. However, HSI and E-nose has still not been exploited for monitoring inactivation programs in the meat industry. The suitability of using HSI and electronic nose to monitor pathogen inactivation effectively was explored in this study. Principal component analysis (PCA) and partial least squares regression (PLSR) machine learning algorithms were applied to HSI spectra and E-nose signals for qualitative and quantitative prediction of pathogen inactivation by ultrasound treatment.

2. Materials and methods

2.1. Pork sample preparation and bacteria inoculation

The fresh longissimus pork meat was bought from a supermarket in Zhenjiang (China) and transported to the laboratory food-grade polymer wraps. Working under hygienic conditions the meat was cut (5 × 5 × 2 cm), in the laboratory. All meat contact surfaces were thoroughly washed and disinfected and place in the hood with ultraviolet germicidal lamp for an hour. Prior to inoculation, the fresh meat samples were tested for Escherichia coli O157: H7 and Salmonella Typhimurium using the microbiology of food and animal feeding stuffs. — Horizontal method for the detection of Escherichia coli O157 (ISO 16654/2001) and Salmonella spp. (ISO 6579/2002) microbial test methods and were determined to be negative in 25 g.

Escherichia coli O157: H7 ATCC 35150 and Salmonella Typhimurium CICC 22956 (Center of Industrial Culture Collection in Beijing, China) was used as target pathogens for this study. In other to provide an objective classification and quantitative study, the choice of bacteria was made to include both gram-positive and negative bacteria. Frozen culture on slant agar was activated in Tryptic Soy Broth (TSB, AOBOX, Beijing) at 37°C for 24 h for 24 h.

The appropriate serial dilutions of fresh Escherichia coli O157: H7 and S. Typhimurium culture, to attain inoculation levels of ~10^7 cfu/ were performed accordingly. The inoculum was thoroughly mixed with the meat samples for three minutes using a sterile spatula. The inoculated meat samples were placed in a refrigerator (4°C) for 30 min to allow bacteria adhesion to the meat surfaces prior to sonication. Samples of uninoculated meat were prepared identically.

2.2. Sonication treatments

A bath-type multi-frequency ultrasound designed by School of Food and Biological Engineering (Jiangsu University, China) fitted with 20, 40, 60 kHz frequency generators with 300 W power setting and 10 and 3 s pulsation on and off time, respectively [12]. In this study, inoculated pork samples (5 g in glass bottles) were subjected to a single 20 kHz frequency for 10, 20, and 30 min, at a water temperature of 4°C. The temperature of the processing water was regulated with a circulating thermostatic water bath.

After sonication was completed, samples were straightaway transferred and prepared for E-nose sampling and imaging, after which they were kept at 4°C for < 45 min before plating was executed.

2.3. Reference enumeration of bacteria

Reference microbial analysis was performed using the plate count method. Selected samples of the inoculated meat before and after ultrasound treatments were removed from the cups into sterile bags containing measured sterile peptone water and mixed with a mixing machine. After the appropriate serial dilutions, bacterial enumeration was carried out according to [13]. Using Sorbitol MacConkey Agar (sorbitol) and Xylose Lysine Deoxycholate Agar (sorbitol) for E. coli O157:H7 strains and Salmonella Typhimurium respectively. Typical colonies were counted after 48 h of incubation at 37°C.

For antimicrobial analysis, the three ultrasound treated as well as a control sample with an initial inoculation of approximately 7log CFU/g was stored at 4°C for five consecutive days. Triplicates were taken out each day for enumeration.

2.4. Kinetic modelling

The kinetic mathematical models defining microbial pathogens inactivation used in this study are shown below. Weibull [14] and log-linear [15] models, as well as a description of the parameters, is described

\[
\text{weibull model } \log N_t = \log N_0 - \left(\frac{t}{\delta}\right)^\rho \delta, \rho
\]

\[
\text{Log - linear } \log N_t = \log N_0 - k. \ \ \text{kwhere}
\]

\[
N_0 \text{ is the initial bacterial population; } N_t \text{ is the number of bacteria that survived after ultrasound treatments; } t \text{ is the ultrasound treatment time; } \delta \text{ is the scale parameter (time for the first decimal reduction); } \rho \text{ dimensionless shape parameter and } k \text{ is the inactivation rate}
\]

Fitting of the kinetic inactivation models was performed using origin-2016 software. The model is evaluated using the root mean square error (RMSE), and the sum of Squares for Error (SSE). Coefficient of determination (R^2), and adjusted R^2.

2.5. Hyperspectral (HSI) imaging acquisition

Each pork sample was imaged using the visible – near-infrared Hyperspectral (HSI) imaging system described by [8,16]. Before spectra data extraction, all raw spectra images were converted into reflectance images using HSI analyser (Spectral Image software, Isuzu Optics Corp., Taiwan, China). spectra data extraction was performed using spectra data extraction.

2.6. Electronic nose acquisition

This study was conducted with an electronic PEN3 nose (Airsense
Analytics GmbH, Schwerin / Germany), consisting of 10 metal–oxide–semiconductor sensors (MOS) with specific volatile selectivity [10,17] describes in detail the sampling, Headspace generation parameters and system settings used in this analysis.

Five (5) grams of the sample was placed in a 20 ml vial has been moved, sealed with a magnetic screw cap for 10 min to ensure the gas generated was concentrated at the top of the sealed bottle prior to analysis. The ten (10) sensor response values were recorded for 120 s. The PEN3 Win Muster v. 1.6.2 program was used to acquire and interpret data.

2.7. Processing and modelling of spectra and signal features from HSI and E-nose

The mean-differential coefficient value was applied to describe the E-nose sensor’s average response velocity and its main characteristics [18]. Therefore, the mean differential coefficient value M(i)ave was taken as the characteristic value of the sensor response curve.

\[
M(i)_{ave} = \frac{1}{n-1} \sum_{i=1}^{n} \frac{x_{iz} + 1 - x_{iz}}{\Delta t}
\]

\[
n = \text{number of test points (} n = 120\)
\]
\[
i = \text{sample number of each variety}
\]
\[
x_{iz} = \text{zth response value of the ith sample}
\]
\[
\Delta t = \text{time difference of adjacent test points (} \Delta t = 1 \text{ s)}
\]

For the HSI spectra (618 variables per each spectra), pretreatment of spectra was conducted before the calibration stage to ensure maximum efficiency. Spectral pretreatment algorithm, standard normal variate (SNV), was applied for scatter correction and noise reduction.

Furthermore, two-hybrid wavelength selection algorithms were applied to select fewer representative variables capable of enhancing predictive performance and reliability of calibration models. The principle of hybrid variable selection is to integrate two techniques; typically, the first algorithm excludes uninformative variables, while the second algorithm further optimises the variables chosen by the previous method. Ant Colony Optimization (ACO) and Genetic Algorithm (GA) [19] was combined with a hybrid variable combination population analysis (VCPA) [20] individually to form a hybrid and applied for spectral selection.

2.8. Qualitative and quantitative analysis

Unsupervised principal component analysis (PCA) was applied to HSI and E-nose datasets to discriminate between raw meat samples and inoculated meat samples subjected to ultrasound treatment. PCA reduces the dimensionality of data sets by transforming a set of correlated variables into a set of new orthogonal variables called principal components (PCs). Linear discriminant analysis (LDA) was also employed to develop a classification model.

Partial Least Squares Regression (PLSR) was applied to predict the colony count of bacteria inoculated meat samples. As a multi-linear regression model, PLSR integrates multiple regression theorem and PCA to build robust and high-performance models [22]. PLSR model is

| Pathogen | sample  | Sonication time | 0 min (inoculated) | 10 min | 20 min | 30 min |
|----------|---------|----------------|--------------------|--------|--------|--------|
| S. Typhimurium | Pork    | 7.35 ± 0.36    | 6.44 ± 0.18        | 5.7 ± 0.13 | 4.1 ± 0.3 |
| E. coli     | pork    | 7.24 ± 0.56    | 6.12 ± 0.22        | 4.3 ± 0.11 | 3.4 ± 0.12 |

Table 2

| Pathogen       | Frequency (kHz) | R² | Adj. R² | RMSE   | K     |
|----------------|----------------|----|---------|--------|-------|
| S. Typhimurium | 20             | 0.9987 | 0.9980 | 0.0723 | 10.3497 1.11507 |
| E. coli        | 20             | 0.9840 | 0.9760 | 0.2354 | 7.48424 0.99163 |

Loglinear model

| Pathogen       | Frequency (kHz) | R² | Adj. R² | RMSE   | K     |
|----------------|----------------|----|---------|--------|-------|
| S. Typhimurium | 20             | 0.9955 | 0.9925 | 0.7564 | 0.2460 |
| E. coli        | 20             | 0.9840 | 0.9840 | 0.0856 | 0.3046 |

Table 1

| Pathogen | sample | Sonication time | 0 min (inoculated) |
|----------|--------|----------------|--------------------|
| S. Typhimurium | Pork    | 7.35 ± 0.36    |
| E. coli     | pork    | 7.24 ± 0.56    |

Fig. 1. Survival and growth of a. S. Typhimurium and b. E. coli during storage of inoculated pork treated by ultrasound.
suited and has been applied severally to solve target prediction problem based on HSI and E-nose datasets [23].

To examine the predictive ability of the models generated and further evaluate the model’s accuracy, the root-mean-squared error of calibration on the calibration set (RMSEC), the root-mean-squared error of cross-validation (RMSECV), the root-mean-squared error of prediction on the prediction set (RMSEP) and their respective Correlation coefficients (R²) as well, the ratio performance deviation (RPD) in the prediction process was also calculated. The PCA, PLSR and variable selection algorithms analysis were run in MATLAB 2018b (The MathWorks Inc., USA).

A total of two-hundred (200) samples per each bacteria group representing forty (40) un-inoculated inoculated, and the three ultrasound treated datasets. SPXY (sample set partitioning based on joint x-y
distances) method [21] was applied to split the acquired E-nose and HSI datasets into training/calibration and prediction sets for classification and quantitative monitoring.

3. Results and discussion

3.1. Microbial inactivation in meat after sonication treatments

Generally, the effect of ultrasound treatment reduced S. Typhimurium and E. coli concentrations considerably in the inoculated pork samples (P < 0.0001). The population of S. Typhimurium and E. coli in the pork samples were approximately 7 ± 0.36 and 7 ± 0.56 log CFU/cm², respectively. A 1–4.3 and 1–4.6 log reduction in S. Typhimurium and E. coli was achieved respectively by sonication treatments ranging from 10 to 30 min. It was also observed that with increased treatment time, particularly to 30 min, the reductions in both pathogens increased significantly. Table 1 shows the mean effect of ultrasound treatment in terms of log reduction on meat inoculated with S. Typhimurium and E. coli at 10, 20 and 30 mins. Significant differences exist between Gram-positive bacteria (S. Typhimurium) and Gram-negative bacteria (E. coli) as suggested in the literature [24,25].

Because of a thicker cell wall due to the coating of peptidoglycans, which defends the cell against sonication, gram-positive bacteria are evidently more resistant to US, while other researchers found no difference in US impact on Gram-positive and Gram-negative bacteria, our study revealed slightly lower log reductions in S. typhimurium than E. coli.

Fig. 4. Frequency of variables selected after 100 runs based on hVCPA preprocessed spectra a. S. Typhimurium and b. E. coli.

Fig. 5. Radar plot of sensor responses S. Typhimurium inoculated pork sample with sonication treatments.
3.2. Bacterial inactivation kinetics and modelling

The kinetics of ultrasound microbial inactivation is experimentally evaluated for *S. Typhimurium* and *E. coli*. The effect of ultrasound inactivation of a microorganism typically follows kinetics of first-order if ultrasound is the single lethal force. The sensitivity of microorganisms to ultrasound treatment varies.

In order to acquire a robust mathematical model, which can correctly represent the bacterial inactivation kinetics and establish the right conditions for sonication treatments [12]. Weibull and Log-linear models successfully described and explained *S. Typhimurium* and *E. coli* inactivation kinetics perfectly based on $R^2$, adj. $R^2$, SSE, and RMSE values described in Table 2. An $R^2$ of 0.9987, 0.9840 (Weibull) and 0.9955, 0.9840 (Loglinear) for *S. Typhimurium* and *E. coli* respectively was reported with a lower RMSE of < 0.00245 for all models.

The Weibull model showed slightly improved results and is consistent with literature and has been commonly implemented in various microbial inactivation assessments of non-thermal technologies due to the simplicity and reliability [14,15,26]. In assessing the kinetic parameters of the Weibull model, when $\rho < 1$ shows cumulative damage caused by cavitation on the microbial cell, rendering the cells more susceptible to lethal treatment, while The higher the $\delta$ value (The first decimal bacterial colony reduction period needed), the slower the microorganism inactivation cycle [26]. From the results, the Weibull kinematic parameters evidently support, the lower inactivation numbers of *S. Typhimurium* at all stages as compared to *E. coli*. The same effect is evident in the log-linear model, where the higher the K value kinetic parameter in the higher the inactivation rate [27].

3.3. Antimicrobial effect of ultrasound treatment

Ultrasound is reported to have a potent antibacterial activity that is effective against a wide range of species [28].

The bactericidal impact of ultrasound treatments in food matrices is mainly due to intracellular cavitation. These micro-mechanical shockwaves disrupt cellular structural and functional constituents, ultimately leading to cell lysis. Additional experiments were performed to demonstrate the bactericidal effect at ultrasound (20 KHz) and to evaluate microbial inactivation at the exposure times (10, 20, 30 min). With an initial inoculum of approximately 7 log CFU/g, *S. Typhimurium* and *E. coli* counts after ultrasound treatment and storing meat for more than 5 days at room temperature are shown in Fig. 1. Bacterial count in the control sample rose from day 1 to > 12.2 and 13.2 log CFU/g at the end of storage (Day 5), while the treatment with ultrasound reduced the amount of bacteria, particularly when longer periods (30 min) of ultrasound treatment were applied.

These results affirm the suitability of ultrasound as an emerging technology to handle pathogen inactivation in meat products and conferring antimicrobial effects without compromising their quality.

3.4. Spectral data and feature analysis

The raw reflectance spectra feature ($n = 200$) of non-inoculated and inoculated pork with *S. Typhimurium* and *E. coli* treated at 20 KHz for 10, 20 and 30 min is shown in Fig. 2a and b, respectively. The baseline drift of the spectrum obtained from this analysis was eliminated through the usage of the Standard Normal Variate (SNV) normalization preprocessing algorithm. While the locations and numbers of peaks and troughs are identical in both preprocessed spectrum information, the wavelength bands of remained significantly thicker in

![Fig. 6. Radar plot of sensor responses *E. coli* inoculated pork sample with sonication treatments.](image-url)
the SNV processed spectrum data (618 variables).

Variable selection algorithm was applied for wavelength reduction [29]. For this study a modified variable combination population analysis (VCPA) designed by Yun, Bin, Liu, Xu, Yan, Cao and Xu [20] which retains 100 variables to be further processed by genetic algorithm (GA) was applied. Fig. 3 shows the flowchart of the modified VCPA for our hybrid strategy. The Hybrid VCPA selected 100 wavelengths for E. coli and S. Typhimurium with RMSECV = 0.388 and 0.2034 respectively. GA (Fig. 4) was then applied to hVCPA selected variables (100) to select 14 and 12 variables of more than 5 and 9 frequencies following 100 runs for E. coli (434.35, 436.83, 437.66, 438.49, 439.32, 440.97, 452.58, 479.24, 499.33, 528.79, 529.64, 541.47, 579.69, 707.85 nm) and S.Typhimurium (437.66, 438.49, 439.32, 440.14, 443.46, 447.60, 457.57, 481.74, 487.60, 538.93, 567.77 nm) respectively. GA parameters genetic iterations, cross-over and mutations probabilities were set at 100, 0.2 and 0.01 respectively.

The rise in pathogen loads has been shown to some degree to influence the spectral details of the meat. This occurrence was predominantly due to varying chemical compositions of fish meat triggered by bacterial infection during storage. Overtone and combination vibrations of molecular chemical bonds correlated with O – H, C – H, C – O, N – H and others are commonly used to understand spectral variations better [30].

Fig. 2a and b show visible and notable absorption peaks at 500–600 nm, which is correlated with absorption of pigments such as astaxanthin in the meat muscle. It is worth noting that most of the optimal wavelengths have fallen within the visual spectrum, possibly due to the fact that the content of astaxanthin, fatty acids protein, present showed some effect on microbial activity.

3.5. Electronic nose data and feature analysis

The average sensor responses of non-inoculated and inoculated pork with S. Typhimurium and E. coli treated at 20 KHz for 10, 20 and 30 min as analysed using ten (10) sensors is shown in Figs. 5 and 6, respectively.

The results of e-nose indicated that several volatile compounds were present in ultrasound treated samples. As represented in Figs. 5 and 6, the relative intensities of volatile compounds changed during the ultrasound treatment process, as seen in the different response patterns.

3.6. Qualitative analysis

E-nose and HSI results revealed apparent differences in ultrasound treated and untreated samples. The digital representation of the variations was, however, difficult to realise. For further research, principal component analysis (PCA) has been used as a chemometric tool to show discrimination ability.

The data points were separated and scattered around the graph after PCA processing for different ultrasound treatments for both sets of data (Fig. 7). The untreated and the treated sample at three-time intervals were widely dispersed and indicated that the E-nose sensor response and HSI spectra data could be used to differentiate between various ultrasound treatments.

The overall rate of contribution of PC1, PC2 and PC3 for e-nose - S. Typhimurium, e-nose -E. coli, HSI - S. Typhimurium and HSI -E. coli was 99.26%,99.63%,99.70%, 99.43% and was considered substantial information to represent all the samples. Since the PCA findings were highly related to treatment times and pathogen inactivation, we further explored the possibility to quantitatively analyse and predict pathogen contamination and reduction by ultrasound treatment.
Linear Discriminant analysis (LDA) algorithm was applied to develop the discriminatory model (200 samples) for e-nose and HSI datasets for both pathogens with training datasets (120 samples) and predictive datasets (80 samples) used for modelling.

The LDA results in Fig. 8 show an excellent overall discrimination accuracies (training set, prediction set) for E-nose - S. Typhimurium (97.50%, 96.25%), E-nose - E. coli (96.70%, 95.65%), HSI - S. Typhimurium (99.17%, 98.75%) and HSI - E. coli (99.17%, 97.50%). There is a slight overlap between two classes, as seen in the confusion matrix (Table 3) particularly between samples treated at 20 KHz@10 min and 20 KHz@20 min. The nulls in the non-diagonal boxes demonstrate that in the vast majority of instances, there are no misclassification. The class-specific sensitivity (% of class instances found correctly) and class-specific precision (% of predicted in-class cases that was correct) is also shown.

The results clearly show the ability of HSI and E-nose to discriminate between the different ultrasound treatments times as well as discriminating between inoculated and un-inoculated samples. The ability to discriminate between contaminated and uncontaminated pork samples is also evident. The results also confirmed results from other E-nose studies by [31–33] on beef and goat meat. No study has been reported yet at the time of this study on HSI discrimination on pathogen contaminated meat.

### 3.7. Quantitative analysis

In estimating bacteria colony counts, the possibility of using the E-nose and HSI datasets from the inoculated pathogen inoculated meat was investigated.

For bacterial quantification, we employed data from four groups, i.e. inoculated samples and the three ultrasound treated datasets (160 samples-40 in each group) with calibration set (96) and prediction set (64). E-nose sensor response and HSI spectral data were modelled against bacterial reference count. High $R^2$, $R^2_{cv}$ and $R^2_p$, and $RPD$ values; and significantly lower RMSEC, RMSECV and RMSEP values are representative of the established model's strong predictive performance, hence the detection and quantification of the inoculate pathogens.

Results from Table 4a shows S. Typhimurium achieved a high $R^2_p = 0.9375$, $RMSEP = 0.2107 \log CFU/g$ and $RPD = 9.7240$ for E-nose HSI showing a slightly improved prediction of $R^2_p = 0.9687$ $RMSEP = 0.1985 \log CFU/g$ and $RPD = 10.3217$ Whereas, values slightly higher than S. Typhimurium was achieved for E. coli for E-nose ($R^2_p = 0.9531$, $RMSEP = 0.2057 \log CFU/g$ and $RPD = 9.9604$) and HSI ($R^2_p = 0.9687$, $RMSEP = 0.2014 \log CFU/g$ and $RPD = 10.1731$).

Overall HSI showed slightly improved results than E-nose, this could be attributed to the application of variable selection algorithms (VCPA-GA) which depends on principal components and their respective lower RMSECVs to determine the option of spectral variables to select from [34].

#### 3.7.1. Validation of model using independent test data

In order to validate this model and ensuring that the model successfully meets its intended function, an independent experimental test data set prepared and preprocessed under the same conditions,
including 45 samples treated at 20 kHz frequency for 10(15 samples), 20(15 samples), and 30(15 samples) minutes. The quantification of regression correlation values (R² values) and Root mean squares error of prediction (RMSEP) was used to measure the multivariate model.

The model based on the HSI provided the best results with Rp² of 0.9526, 0.9625 and RMSEP of 0.1452, 0.1865 particularly for predicting for E. coli and S. Typhimurium contaminant levels after sonication treatment in the testing set as compared with their corresponding E-nose test data set (Table 4b). The estimated RPD value of 11.254 and 9.9531 for the VCPA-GA HSI based model indicates its robustness and superiority over the E-nose PLS model with better prediction capacity.

Evidently, the predictive performance of prediction set was higher than that of independent testing data, presumably because the samples attributed to testing data did not belong to the same set and were not used in the calibration model development at all [35].

Table 3
Confusion matrix using LDA (training set size = 120, Prediction set size = 80).

|          | E. coli - S. Typhimurium | S. Typhimurium | E. coli |
|----------|--------------------------|----------------|--------|
| No. of samples | Classification Results | Sensitivity% | Precision% | No. of samples | Classification Results | Sensitivity% | Precision% |
| A        | B          | C        | D        | E        | A    | B    | C        | D        | E        | A    | B    | C        | D        | E        |
| 24       | A          | 24     | 0      | 0      | 0     | 100  | 100   | 0          | 0      | 0     | 16   | 100  | 100   |
| 24       | B          | 0      | 24     | 0      | 0     | 100  | 96    | 100       | 0      | 0     | 16   | 100  | 100   |
| 24       | C          | 0      | 0      | 24     | 0     | 100  | 100   | 0          | 23     | 0     | 16   | 100  | 94.11 |
| 24       | D          | 0      | 0      | 0      | 24    | 100  | 100   | 0          | 0      | 0     | 16   | 100  | 100   |
| 24       | E          | 0      | 0      | 0      | 0     | 24   | 100   | 0          | 0      | 0     | 16   | 100  | 100   |

Table 4a
Results of different variable selection methods on the E. coli and S. Typhimurium contaminated pork spectra dataset.

| Pathogen | Method | RMSEC | RMSECV | RMSEP | R² | R² | RPD |
|----------|--------|-------|--------|-------|----|----|-----|
| S. Typhimurium | E-nose | 0.3754 | 0.3972 | 0.2107 | 0.9640 | 0.9613 | 0.9375 | 9.7240 |
| S. Typhimurium | HSI    | 0.2502 | 0.2824 | 0.1985 | 0.9895 | 0.9756 | 0.9687 | 10.3217 |
| E. coli     | E-nose | 0.3707 | 0.4074 | 0.2057 | 0.9680 | 0.9605 | 0.9531 | 9.9604 |
| E. coli     | HSI    | 0.2735 | 0.2865 | 0.2014 | 0.9791 | 0.9652 | 0.9687 | 10.1731 |

Table 4b
Results of Validation of model using independent test data on the E. coli and S. Typhimurium contaminated pork spectra.

| Pathogen | Method | RMSEP | R² | RPD |
|----------|--------|-------|----|-----|
| S. Typhimurium | E-nose | 0.2415 | 0.9123 | 8.5485 |
| S. Typhimurium | HSI    | 0.1452 | 0.9526 | 11.254 |
| E. coli     | E-nose | 0.2665 | 0.9321 | 8.4529 |
| E. coli     | HSI    | 0.1865 | 0.9625 | 9.9531 |
4. Conclusion

The findings of this research showed that treatment with ultrasound at 20 KHz for 10, 20 and 30 min resulted in reduced rates of S. Typhimurium and E. Coli in the inoculated pork sample. Ultrasound treatment can be deemed an appropriate method for preservation and storage of meat products under the measured conditions. We validate that the Weibull model is ideally fit for formulating food preservation recommendations or food safety processing requirements [27].

We also show for the first time, nondestructive monitoring of ultrasound inactivation of bacteria foodborne pathogens by E-nose and HSI Both E-nose and HSI designed PLS models achieved excellent predictive efficiency with respect to the estimation of E. coli and S. Typhimurium concentrations in inoculated pork samples with their respective RPDs above 3. The RPD is a statistical data often used measure calibration model's predictive accuracy. It is measured as standard deviation (SD) of the sample data to either RMSECV or RMSEP. Based on literature review [36], an RPD meaning may be proposed for five separate degrees of predictive accuracy. A higher RPD > 3 indicates the technique has an exceptional predictive potential.

Subsequently, a theoretical foundation has been developed for optimizing and reducing the number of variables of HSI dataset by hybrid wavelength variable selection algorithms with comparatively lower errors.

The results from our study show that the nondestructive E-nose and HSI approach is an effective quantitative technique for estimation and monitoring of bacterial foodborne pathogen count during inactivation mechanisms like ultrasound.

Nevertheless, more work is needed to attain conditions that preclude bacteria from re-growing, attain complete inactivation during storage, and validate whether the effects of re-growth originate from damaged cells.

CRediT authorship contribution statement

Ernest Bonah: Conceptualization, Data curation, Writing - original draft. Xingyi Huang: Funding acquisition, Resources, Investigation, Writing - review & editing. Yang Hongying: Methodology. Joshua Harrington Aheto: Writing - review & editing. Ren Yi: Software, Validation. Shanshan Yu: Investigation. Hongyang Tu: Visualization.

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.

Acknowledgement

This work was supported by the National Key Research and Development Program of China (No. 2017YFD0400102).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2020.105344.

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