Emerging applications of nano-optical sensors combined with near-infrared spectroscopy for detecting tea extract fermentation aroma under ultrasound-assisted sonication

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ABSTRACT

The current innovative work combines nano-optical sensors with near-infrared spectroscopy for rapid detection and quantification of polyphenols and investigates the potential of the nano-optical sensor based on chemo-selective colorants to detect the dynamic changes in aroma components during the fermentation of tea extract. The procedure examined the influence of different ultrasound-assisted sonication factors on the changes in the consumption rate of polyphenols during the fermentation of tea extract versus non-sonication as a control group. The results showed that the polyphenol consumption rate improved under the ultrasound conditions of 28 kHz ultrasound frequency, 24 min treatment time, and 40 W/L ultrasonic power density. The metal-organic framework based nano-optical sensors reported here have more adsorption sites for enhanced adsorption of the volatile organic compounds. The polystyrene-acrylic microstructure offered specific surface area for the reactants. Besides, the employed porous silica nanospheres with higher porosity administered improved gas enrichment effect. The nano-optical sensor exhibits good performance with a “chromatogram” for the identification of aroma components in the fermentation process of tea extract. The proposed method respectively enhanced the consumption rate of polyphenol by 35.57%, 11.34% and 16.09% under the optimized conditions. Based on the established polyphenol quantitative prediction models, this work demonstrated the feasibility of using a nano-optical sensor to perform in-situ imaging of the fermentation degree of tea extracts subjected to ultrasonic treatment.

1. Introduction

The intensive processing of summer and autumn tea is an important direction to follow for the efficient use of the tea resources. The fermentation and processing of instant tea extract can maximize the high-value utilization of low-value summer and autumn tea resources [1,2]. Fermentation is considered critical in the aroma origin. It is in essence, an enzymatic oxidation reaction, wherein various polyphenols generate a large number of compounds such as alcohols, aldehydes, and acids [3–5]. The efficiency of the tea fermentation process is governed by the reaction speed. The cell wall and cell membrane’s rupture induce the corresponding flavor substances to overflow in the enzymatic oxidation reaction. Thus, the fermented tea has a unique color, aroma, and taste [6,7].

The current tea fermentation strategies are primarily traditional in origin and involve numerous complications. The enzymatic oxidation reaction during the fermentation process is greatly governed by external factors which influence the aroma of tea. Therefore, it is necessary to design new tea fermentation modes to improve the efficiency of the process and enhance the aroma quality [8,9]. In recent years, with the interdisciplinary integration of physics, chemistry, and biology, there has been an increased interest in the applications of ultrasound technology in the field of food processing [10]. Tizazu et al., [11] examined ultrasound-assisted xyitol production through fermentation of dilute acid (pentose-rich) hydrolysate of sugarcane bagasse using free cells of Candida tropicalis. Ultrasound technology has also been used to decrease the aging process of vinegar. To further demonstrate its ability to accelerate the chemical reaction of crabapple vinegar, ultrasonic...
The spectra data was then obtained using near-infrared spectroscopy (NIRS), the optimal spectral region was divided and modeled by collaborative interval partial least squares (SIPLS), based on ant colony optimization (ACO-PLS), genetic algorithm-PLS (GA-PLS) and competitive adaptive reweighted sampling-PLS (CARS-PLS) to predict the consumption rate of tea polyphenols during the ultrasonic fermentation of tea. This study explored the changes of aroma substances in the fermentation process of tea extract under ultrasonic field stress and implemented in-situ imaging in real-time.

2. Materials and methods

2.1. Experimental material

The green tea powder was provided by Nanjing Rongdian Food Technology Co., Ltd. The kombucha was purchased from Anqing Min- gxin Tosen Trading Co., Ltd. (Anhui, China). Phosphate Buffered Saline (PBS) was supplied by Macklin (Shanghai, China). The water used in the whole experiment was Millipore Milli-Q ultrapure water. Anthrone was purchased from Shanghai Wokai Chemical Reagent Co., Ltd., and Folin, concentrated sulfuric acid and sodium carbonate were purchased from Shanghai Test Laboratory Equipment Co., Ltd. All chemicals and reagents used were of analytical grade and were used without further processing.

2.2. Software

The data were collected and processed in HP-Chemstation System Workstation and compared with NIST17. Firstly, the raw GC-MS data was integrated by GCMS solutions software (V 4.52, SIMADZU, Japan). The integration conditions were set as follows: the initial peak number was kept at 5, and the slope and variable parameter time were set as 600/min and 1000 min, respectively. Next, the data obtained was registered to the NIST17 Standard Mass Spectrometry Database (NIST, Gaithersburg, Maryland, USA) for identification of metabolites from the selected variables. The features with similarity (SI) less than 50% in the sample were removed. MetaboAnalyst (https://www.metaboanalyst.ca) is a comprehensive network analysis platform for quantitative metabolomic data [40].

2.3. Experimental set-up

2.3.1. Mother liquor preparation

A sucrose medium with a ratio of sucrose and distilled water in 1:10 was formulated for kombucha fermentation. The tea fungus (diameter 10 cm, thickness 1 cm), appropriate amount of bacteria liquid and culture medium were placed in a 2 L glass bottle covered with gauze, and fermented at room temperature for one week.

2.3.2. Sonication factors experiment

To manufacture and evaluate the tea extract fermentation, a bioreactor and an aroma detector comprised of a fermentation tank, a sonication device, and a near-infrared detection system were used in this study. (Fig. 2). This device is primarily composed of 21 parts, i.e., 1. slit ultrasonic equipment, 2. control panel, 3. ultrasonic generator, 4. ultrasonic transducer, 5. material liquid inlet, 6. material liquid outlet, 7. sterilization vent, 8. peristaltic pump, 9. fermentation tank, 10. circulating condensate, 11. fermentation tank main console, 12. pH electrode, 13. temperature sensor, 14. data cable, 15. air filter, 16. Spectrometer, 17. detection slot, 18. shading cover, 19. optical cable, 20. light source and 21. computer.

The bioreactor was operated according to the following procedure: The fermentor was filled with a 2 L tea extract and 200 mL of kombucha...
A 2 L tea extract with 200 mL kombucha fluid was added to the fermentor. Under a constant ultrasonic power density (100 W/L) for ultrasonic peristalsis (30 min), the sonication treatments were utilized to stimulate kombucha fermentation and the effects of different ultrasonic frequencies (20, 23, 25, 28, 33 and 40 kHz) on the consumption rate of polyphenols during the fermentation of tea extract were investigated. The ultrasonic time in the experimental group was examined at different time intervals of 6, 12, 24, 48 and 60 min. Different power densities of 20, 40, 60, 80, and 100 W/L were designed for the single factor optimization experiment of ultrasonic power density. The stirring speed was set at 150 rpm (28 °C) in the fermentation tank’s main console, and the device was started by the control panel. The conditions of 30 °C of fermentation temperature, natural pH, inoculation amount 10% were applied in the experiment. NIR spectroscopy was used to measure the spectral transmittance by the optical fiber transmission method with an optical path of 10 mm.

2.3.3. Determination of polyphenols

The polyphenol values of tea extract fermentation broth were determined daily in accordance with national standard method (GB/T 21733–2008). Accurately 2 g of fermentation broth was weighed into a 25 mL volumetric flask. This is followed by addition of 4 mL of water, 5 mL of ferrous tartrate solution, and the solution mixture was completed to the 25.0 mL mark with PBS buffer. A 10 mm cuvette was used to determine the absorbance (A1) with reagent blank values deducted at 540 nm. The reagent blank solution was prepared by transferring an accurately weighed (2 g) fermentation broth into a 25 mL volumetric flask, followed by 4 mL of water, and the volume was adjusted to 25 mL with PBS buffer. The absorbance (A2) was measured after subtracting the reagent blank. The equation for calculating the content of tea polyphenols was as follows:

\[ X = \frac{(A1 - A2) \times 1.957 \times 2 \times K \times 1000}{m} \]

where \( X \) is tea polyphenols content in the sample (mg/kg); \( A1 \) is absorbance of test solution after color development; \( A2 \) is absorbance of the background color of the test solution; 1.957 integer represents the condition at 0.50 absorbance where the content of polyphenols in 1 mL of tea infusion is equivalent to 1.957 mg; \( K \) is dilution times; \( m \) is weigh the mass of the test solution during measurement (g).

2.4. Measurement of VOCs indices

The VOCs were determined by accurately taking 6 mL of tea extract fermentation broth from the fermentation tank every 12 h, in triplicate. The extraction and sample injection analysis processes were automated. The injector penetration with a depth of 54 mm was used, and the SPMEArrowCond1 was selected as a conditioning port. SPME extraction was developed and modified based on methods reported in ref [41]. Tea extract samples (6 mL) were weighed and placed in a 20 mL screw-capped headspace vial. Subsequently, 6 μL of internal standard (ethyl decanoate, 10 μg/mL) was introduced through a micro-injector [42]. The HS-SPME extraction procedure was carried out using the AOC-6000 autosampler. A SPME fiber was inserted into the sample headspace and allowed to stircontinuously at 250 rpm for 30.0 min at 70 °C. After extraction, the fiber was removed from the vial and immediately inserted into the GC–MS sampler for desorption (250 °C for 5 min) and further analysis.
Triple quadrupole gas chromatography-mass spectrometer (TQ-8040) was used for GC–MS analysis. An Rtx-5MS (30 m × 0.25 mm × 0.25 μm) was used as the column, with high-purity helium, as the carrier gas at a flow rate of 1 mL/min. Temperature rise procedure: Initial temperature was noted as 50 °C, held for 4 min, and then increase to 290 °C at the ratio of 6 °C/min, held for 5 min, and finally increased to 310 °C at the ratio of 10 °C/min and held for 5 min [36]. The MS ion source temperature and the inlet temperature were 230 °C and 250 °C, respectively, and the electron energy was 70 eV [43]. The scanning range was 35–550 atomic mass unit (amu).

More parameter settings of the sampler are shown in Table 1. The SPME extraction head was inserted into the GC–MS sample inlet after extraction. Chromatographic conditions and mass spectrometry conditions are also listed in Table 1.

2.5. Data analysis

The spectral data of 120 samples obtained for the different fermentation stages (fermented times of 0, 12, 24, 36, 48, 60, 72 and 84 h) were divided into two groups of the training set and prediction set in a 2:1 ratio for monitoring the ultrasonic fermentation process of tea extract. In addition to the sample-related information, the NIR original spectrum contains noise and redundancy, especially the baseline drift, instrument errors, environmental conditions, etc., [18,44]. This might lead to significant interference in the spectrum and influence the quantitative prediction of the active components of the sample. Therefore, the data were subjected to quantitative prediction models. Spectral preprocessing, such as the standard normal variable (SNV) algorithm was initially employed to improve the accuracy and stability of the model. The equation of the SNV algorithm is shown below:

$$x_{ik,\text{SNV}} = \frac{x_{ik} - \bar{x}_i}{\sqrt{\frac{1}{m} \sum_{k=1}^{m} (x_{ik} - \bar{x}_i)^2}}$$

(1)

Where $x_{ik,\text{SNV}}$ represents the value of the spectral data of the $i^{th}$ ($i = 1, 2, ..., n$) samples at the $k^{th}$ ($k = 1, 2, ..., m$) wavenumber points after SNV processing; $x_{ik}$ represents the spectral data of the $i^{th}$ sample at the $k^{th}$ wave point; $\bar{x}_i$ represents the average of the spectral data of the $i^{th}$ sample at all $m$ wave-number points. The calculation Equations for $R^2$ and lower root mean square error of prediction (RMSEP):

$$R^2 = 1 - \frac{\sum_{i=1}^{n_p} (\hat{y}_i - y_i)^2}{\sum_{i=1}^{n} (y_i - \bar{y})^2}$$

(2)

$$\text{RMSE} = \sqrt{\frac{1}{n_p} \sum_{i=1}^{n_p} (y_i - \hat{y}_i)^2}$$

(3)

Where $n_p$ is the number of samples in the prediction set, $y_{ik}$ is the
reference measurement value of the $i^{th}$ sample, $\bar{y}_i$ is the estimated value of the $i^{th}$ sample, and $\bar{y}$ is the average of all reference measurement values in the prediction set.

Partial least squares (PLS) was studied in order to create the models and predict the quality indices through the sensor array responses [45]. PLS is the "gold standard" in chemometrics due to its ability to handle collinear data and reduce the number of required calibration samples [46]. PLS is a fixed linear regression technique of the type $Y = AX + B$, which reduces the size of variables by extracting linear combinations from the original $X$. These combinations are called orthogonal latent components (A). As a supervised technique, it is critical to include a set of validation data in order to select the optimal number of latent variables. The performances of the built models were evaluated by the determination coefficient ($R^2$) and root mean squared error (RMSE) which were calculated through interactive verification. In this work, the spectral data were preprocessed by SNV, which follows the application of chemometrics like PLS. The preprocessed spectral data was then divided by the synergy interval (Si) algorithm to obtain optimal spectral regions. Chemometrics methods, such as Si-ant colony optimization-Si-PLS (Si-ACO-PLS), Si-genetic algorithm-PLS (Si-GA-PLS), and Si-competitive adaptive reweighted sampling-PLS (Si-CARS-PLS) were used to build the regression model based on the chosen spectral regions [47, 48].

3. Results and discussion

3.1. Optimization of ultrasonic frequency, time and power density

During the tea extract fermentation, the production of metabolic waste could damage the strains and inhibit the synthesis of aroma [49, 50], Dahroud et al., [16] and Bundhoo et al., [51] concluded that low-intensity ultrasound can change the activity of related enzymes and increase the permeability of cell membrane. Therefore, this study would explore the fermentation process of tea extract under different ultrasonic conditions and its influence on the changes in the tea polyphenols.

The consumption rate of polyphenol under different ultrasonic frequency conditions is shown in Fig. 3a. It was demonstrated that a significant difference (P less than 0.05) existed in the polyphenol content between non-sonicated and sonicated samples. After 5 days of fermentation of tea extract, the consumption rate of polyphenol without sonication reached 36.60% and the consumption reached a maximum value of 49.62% at 23 kHz of ultrasonic frequency. Sonication treatment (23 kHz) enhanced the consumption rate of polyphenol by 35.53% in comparison to the control group. The polyphenol consumption of tea extract from 23, 25, 33 and 40 kHz experimental groups essentially reached maximum on the fourth day of the fermentation. The content remained unchanged at the end of the fourth day, while the consumption rate of polyphenol increased significantly under ultrasonic frequencies of 20 kHz and 23 kHz. In particular, the consumption rate at 23 kHz was found to be higher than that of 20 kHz, and was set as the optimal ultrasonic frequency. Ultrasound treatment accelerated the fermentation of tea extract in the presence of kombucha, which may be related to the fact that stress responses produced by external stimulation results in the increased polyphenol oxidase activity in the cell, which then accelerates the enzymatic oxidation process [10, 52]. The frequency of 23 kHz was selected with an optimal ultrasonic time of 24 min (as shown in Fig. 3b) and an ultrasonic power density of 40 W/L (as shown in Fig. 3c) were successively explored as the optimal technological conditions for the fermentation process. A certain amount of ultrasound treatment of tea extracts can increase the surface area of the substrate or enhance the cell membrane permeability, thereby accelerating the reaction rate. However, prolonged and excessive ultrasonication may reduce the likelihood of polyphenol oxidase in contact with the substrate and may destroy some flavor compounds, resulting in undesirable results. Ultrasound accelerates the maturation of the fermented food flavors, and the selection of appropriate process conditions is critical for enhancing the tea fermentation process.

3.2. Analysis of aroma of tea extract under ultrasonic fermentation

The aroma of tea extract is mainly formed during the fermentation process under ultrasonic treatment. The floral and fruity aroma of kombucha was mainly contributed by benzyl alcohol and phenylethyl alcohol, which belong to aromatic alcohols with benzene rings [53]. The fermented samples were analyzed by GC–MS. Table 2 summarizes the results of an analysis of the absolute volatile aroma content of tea extracts during ultrasonic treatment and fermentation. After ultrasonic treatment, the fermentation process of the tea extract produces a mixture of 17 different hydrocarbons, 9 of which are alkanes. Alcohols play a significant role in aroma composition due to the fact that there are primarily ten alcohols. Before fermentation, the average concentration of geraniol in tea extract is 19.57 g/L. Geraniol is metabolized to 9.02 g/L after 72 h of fermentation in the fermenter following ultrasonic treatment. Linalool contributed greatly to the floral aroma and was the most abundant aroma component in the early stage of the process [54], but its content declined and varied in the middle stages of the fermentation. The content of linalool showed an increasing trend initially, but then its concentration decreased during the fermentation process. The content of linalool in the tea extract after 3 days of fermentation increased by 35.53%. A total five kinds of main ketones and two kinds of main ethers were respectively detected from tea extract samples. Esters are aroma components that are mainly 3-hexene isovalerate, methyl salicylate, phenethyl acetate, 2-ethylhexyl salicylate, and their content first increased and later reduced. Methyl salicylate is the key aroma

![Fig. 3. The effect of different ultrasonic frequencies (a), ultrasonic time (b) and power density (c) on polyphenol consumption rate.](image-url)
| Absolute content of aroma (g/L) | Fermentation time | 0 h | 3 h | 6 h | 12 h | 24 h | 36 h | 48 h | 60 h | 72 h |
|---------------------------------|------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|                                |                  | 1   | 2   | 3   | 1   | 2   | 3   | 1   | 2   | 3   |
| **Alkanes**                     |                  |     |     |     |     |     |     |     |     |     |
| Undecane                        | 0.39             | 0.28| 0.62| 0.20| 0.66| 0.48| 0.92| 0.76| 1.14| 1.33|
| Dodecane                        | 1.07             | 0.45| 0.84| 0.45| 0.00| 1.01| 0.94| 0.51| 0.00| 1.08|
| Eicosane                        | 0.35             | 1.81| 0.51| 2.19| 0.29| 0.34| 0.56| 0.51| 0.00| 3.32|
| 1,3,6-Octatriene                 | 0.00             | 0.39| 1.29| 0.68| 1.05| 1.71| 0.31| 1.33| 1.27| 1.89|
| Cyclodecane                     | 0.00             | 0.00| 0.00| 0.00| 0.00| 0.01| 0.00| 0.01| 0.29| 0.26|
| Pentadecane                     | 0.00             | 1.11| 0.79| 1.08| 0.00| 0.49| 0.97| 0.00| 4.30| 1.18|
| Tetradecane                     | 0.51             | 0.53| 0.61| 0.90| 0.76| 1.86| 1.85| 1.54| 3.18| 2.02|
| Heptadecane                     | 0.96             | 0.91| 0.85| 1.08| 1.59| 4.61| 3.47| 3.40| 0.00| 6.07|
| Heneicosane                     | 1.09             | 0.99| 1.86| 0.42| 0.29| 2.16| 4.57| 3.15| 2.58| 0.00|
| **Olefins**                     |                  |     |     |     |     |     |     |     |     |     |
| -beta-Mycene                    | 0.02             | 1.79| 5.83| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 5.96|
| 1-Nonadecene                    | 0.87             | 0.81| 0.81| 0.88| 0.50| 0.33| 0.68| 1.12| 0.83| 0.49|
| 1,3,6-Octatriene                 | 0.45             | 0.39| 1.29| 0.68| 1.05| 1.71| 0.31| 1.33| 1.27| 1.89|
| trans-beta-Ocimene              | 0.34             | 0.27| 0.87| 0.45| 0.75| 1.82| 1.00| 1.06| 1.26| 1.43|
| Terpinolene                     | 0.01             | 0.07| 0.33| 0.11| 0.27| 0.24| 0.33| 1.58| 0.42| 0.65|
| Cyclohexene                     | 0.00             | 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.18| 0.05|
| Di-Limonene                     | 0.59             | 0.51| 1.73| 1.19| 1.98| 3.17| 2.68| 2.79| 2.71| 3.95|
| **Alcohols**                    |                  |     |     |     |     |     |     |     |     |     |
| Benzyl alcohol                  | 0.00             | 0.16| 0.52| 0.29| 0.41| 0.00| 0.57| 0.54| 0.00| 0.78|
| Ethanol                         | 0.00             | 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 0.00| 4.93|
| Geraniol                        | 20.39            | 17.90| 20.43| 23.21| 22.35| 27.63| 19.09| 18.98| 18.39| 26.74|
| Terpenen-4-ol                   | 0.19             | 0.10| 0.14| 0.10| 0.13| 0.23| 0.18| 0.19| 0.00| 0.00|
| Phenylthethyl Alcohol           | 3.48             | 3.90| 2.37| 1.32| 1.38| 3.18| 2.94| 3.22| 3.72| 4.93|
| n-Pentadecanol                  | 0.61             | 0.67| 0.62| 0.83| 0.45| 1.24| 1.15| 1.14| 2.27| 0.00|
| Linalool                        | 30.05            | 28.31| 29.63| 18.99| 26.45| 45.77| 36.31| 37.00| 38.78| 51.65|
| **Acids**                       |                  |     |     |     |     |     |     |     |     |     |
| Octanoic acid                   | 0.00             | 0.00| 0.00| 0.00| 0.00| 0.00| 1.74| 1.54| 1.37| 2.81|
| **Esters**                      |                  |     |     |     |     |     |     |     |     |     |
| Methyl salicylate               | 0.70             | 0.30| 0.45| 0.08| 0.11| 0.19| 0.12| 0.23| 0.14| 0.49|

(continued on next page)
The change of 3-hexene isovalerate has scientific research value in the aroma exploration produced during the fermentation process, with the declining concentration by 71.05%. Despite the presence of a few aldehyde types, octanal and phenylacetalddehyde, in the whole fermentation stage, there are mainly 58 substances detected, as shown in Fig. S1 in the supplementary materials. Octanal and nonanal were saturated aldehydes known as fatty acid oxidation products. Aromatic aldehydes were mainly 2-methylbenzaldehyde and benzaldehyde. Phenylalanine can be pyrolyzed to produce benzaldehyde under higher temperature and humidity conditions [56]. It was found that the content of six kinds of geraniol, linalool, benzaldehyde, nonanoic acid, ether and 3-hexene isovalerate is relatively large and changes with the fermentation time. The changes in the content of the above-mentioned 6 substances of the tea extract during ultrasonic fermentation within 72 h were further analyzed, as shown in Fig. S2. GC–MS analysis results explored geraniol, linalool and 3-hexene isovalerate as important components that constitute the characteristic aroma of tea extract during the ultrasonic fermentation process.

### 3.3. Preparation of Nano-optical sensor to aroma response

#### 3.3.1. Chemo-selective colorants response

Optical sensors are classified according to the nature of their transduction mechanisms. The quantitative measurement of absorbance or reflectance is referred to as optical chemical sensing. In this work, optical chemical sensors use visible light to probe chemical interactions at liquid interfaces (tea extract fermentation broth). Porphyrins are the classic chemo-responsive materials [57]. BODIPY dye employed in this work, which is an intermediate in the synthesis of porphyrin dye [58], is subject to small steric repression and has been proved to be sensitive to aldehydes, ketones, alcohols and other VOCs [39]. The optical chemical sensor detects and captures changes in the aroma of tea extracts during the fermentation process following ultrasonic treatment, and is composed of 24 kinds of synthesized chemical materials (as shown in Fig. 1). To simplify data analysis and instrumentation, the RGB color imaging method was employed to analyze spectra wavelengths. Chemo-selective colorants with a good response to aroma characteristics (geraniol, linalool and 3-hexene isovalerate) were screened as shown in Fig. 4. The color difference ($\Delta R$, $\Delta G$ and $\Delta B$) of linalool with a concentration of 100 ppm before and after exposure to 24-element sensor array of chemo-selective colorants is calculated as shown in Fig. 4b. In comparison to other compounds, the response of #7 to linalool was more pronounced. The chemo-selective colorants for #7, i.e. 8-(4-nitrophenyl)-4,4-difluoro-2,6-dibromoborodipyrrole methane, abbreviated as NO$_2$Br$_2$BDP, are sensitive to linalool that is a characteristic aroma component during the fermentation of tea extract. According to Fig. 4c and Fig. 4d the sensitive material of 3-hexenylisovalerate is bis[8-phenyldipyromethene]nickel (II) (HBDP)$_2$Ni(II)), whereas geraniol is sensitive to bis[8-(4-carbazolephenyl) dipyromethene] nickel (pCarBDP). In practical applications, the sensor constructed from these chemo-selective colorants is not very specific for small VOCs with similar structures and has reduced sensitivity at lower concentrations. With the advances in nanotechnology, novel sensors incorporating nanomaterials have been applied for the detection of aroma substances in order to overcome the limitations mentioned previously.

#### 3.3.2. Characterization of nano-optical sensor

The surface microstructure of polystyrene-acrylic (PSA) helps to increase the specific surface area of the reactants. The high-porosity gas enrichment effect of PSN enhances the material’s adsorption. PSNs were synthesized with CTAB as a modifier (surfactant and porogen). According to Fang et al. [59], PSA was synthesized using a soap-free emulsion copolymerization process with styrene and acrylic acid as monomers in a non-buffered medium, and ammonium persulfate as the initiator. The MOFs have more adsorption sites to improve material
selectivity. Typical transmission electron microscope (TEM) images of the MOF, PSN and PSA are shown in Fig. 5 a, Fig. 5 b and Fig. 5 c, respectively.

As shown in Fig. 5 a, MOFs are rod-like superimposed structures as evident in the TEM data. The structure of PSN and PSA nanoparticles was spherical. TEM analyses were carried out to observe the shape and size of these nanoparticles and to collect the representative images. As can be seen, the particle sizes of these two nanomaterials are uniform, with PSA and PSN having particle sizes of 285 nm and 335 nm, respectively, as measured by Image Pro Plus 6.0 software [60]. Fig. 5 b depicts PSA as a smooth nanosphere, and PSN as a porous nanosphere in Fig. 5 c.

The three kinds of nanomaterials are respectively modified by the chemo-selective colorant dyes (NO$_2$Br$_2$BDP, pCarBDP and (HBDP)$_2$Ni(II)) screened out in Section 3.3.1. The synthesized nanomaterials modified chemo-selective colorant dyes indifferent combinations exhibited distinct UV–Visible absorption spectra. As shown in Fig. 5 d, the highest absorption value of the combinations of PSN modified...
NO$_2$Br$_2$BDP (NO$_2$Br$_2$BDP@PSN) was found to be higher than that of MOF modified NO$_2$Br$_2$BDP (NO$_2$Br$_2$BDP@MOF) and PSA modified NO$_2$Br$_2$BDP (NO$_2$Br$_2$BDP@PSA). However, as compared to the other two combinations, NO$_2$Br$_2$BDP@MOF has a larger band to reach the highest absorption peak. These characterization results also demonstrated successful surface functionalization. Furthermore, various combinations of chemo-selective colorants-modified nanomaterials are provided for optimizing performance under aroma exposure.

3.4. Confirmation in ultrasonic fermentation process detection

3.4.1. Identification of aroma in nano-optical sensor

Image processing collects information about tea leaves such as color, texture, and shape. Machine vision has been proven in studies to elucidate the quality of black tea processing [61,62]. The changes in color and flavor are related to the complexity of the black tea fermentation process, therefore it is difficult for a single sensor to describe the sample information comprehensively and accurately. An array probes a range of analyte – sensor interactions to generate a wide span of molecular specificity based on the chemical properties of sensor elements. There are highly selective sensors that respond exclusively to one analyte, or perhaps one closely related class of analytes. Despite these receptors’ ability to selectively bind with a specific target analyte, they cannot form a sensor array that enables the recognition of other groups of analytes and mixtures. Moreover, the design and synthesis of such specific sensors can be time-consuming and complicated. Thus, the optimal optical sensor array will incorporate a range of colorimetric sensor elements with diverse specificities and chemical interactions. Therefore, the designed nano-optical sensor comprises 12 array elements for the identification of aroma components in the fermentation process of the tea extract (Fig. 6a). These sensor arrays were exposed to tea extract fermentation broth with different fermented times (for 12, 24, 36, 48, 60, 72, 84 and 96 h). The RGB differences were calculated afterward and normalized to 0–255 and their characteristic values were calculated by Equation (4), and the difference image, as a “chromatogram,” was drawn using MATLAB software.

Characteristic values $= \sqrt{3 \times (R^2 + G^2 + B^2)}$ (4)

The “chromatogram” drawn by the characteristic value shows that the sensor elements are composed of different chemo-selective colorants with varying degrees of ability to distinguish the fermented aroma of tea extract. As shown in Fig. 6b, No. 8 and No. 9 elements are the best-performing in distinguishing the aroma substances of tea extracts from different fermentation batches (the color band is the darkest in the color map). Additionally, it was observed that the sensor’s response to the tea extract during the fermentation process is consistent and reproducible at various fermentation times. Generally, the types and contents of flavor substances produced by the tea extract following ultrasonic treatment continue to increase, resulting in a large color response value and a darker color band during the late fermentation period. The “chromatogram” of the sensor, which is based on the R color component and used to distinguish the aroma change of tea extract during fermentation, also verifies the feasibility of the nano-optical sensor to detect volatile substances (the aroma of tea extract). As illustrated in Fig. 6c, Fig. 6d and Fig. 6e, the R color response to the tea extract within 190 to 225 confined to the color band under different fermentation times varied from deep to light, and the G and B response values vary from 150 to

Fig. 6. The nano-optical sensor consisted of 12 array elements (a). The ‘chromatogram’ obtained by characteristic responses (b), R color difference (c), G color difference (d), B color difference (e) of nano-optical sensor to tea extract aroma during the fermentation process.
215. Even though the R, G and B color response variation ranges are smaller than the characteristic variation values (285 to 360), they are capable of capturing the dynamic changes of aroma in the tea extract during the fermentation process.

3.4.2. Prediction of quality indices
To investigate the nano-optical sensor potential for detection of tea extract fermentation aroma, an innovative procedure was performed that combined the potential of the sensors with NIR spectroscopy for rapid detection and quantification of polyphenols. In summary, a novel optical sensor was designed using surface-functionalized chemo-selective colorants as the basic element. For spectral pre-treatment of NIR spectroscopy data, SNV preprocessing tool was used. Following PLS, the data were subjected to the SI-PLS algorithm, which was used to capture the relevant informative variables at the spectral interval for effective quantification. Variable selection chemometrics methods [47], such as ACO-PLS, GA-PLS and CARS-PLS with good modeling performances, have been applied for quantifying the tea polyphenols contents in samples with different concentration gradients [48]. Afterward, ACO-PLS, GA-PLS and CARS-PLS were comparatively applied to further screen variables to construct an optimal prediction model for polyphenols content of tea extract during the fermentation process.

In general, a higher correlation coefficient for the prediction set (Rp) and a RMSEP corresponds to more reliable model results. Therefore, the model based on GA-PLS has the best performance as shown in Fig. 7a with Rp values of 0.9988, and RMSEP values of 0.0264 mg/kg. The results of the nano-optical sensor model based on CARS-PLS and ACO-PLS algorithms are shown in Fig. 7b and Fig. 7c. To further verify the applicability, the proposed method was validated by the national standard method (GB/T 21,733–2008) for detecting tea polyphenols as seen in Table 3. The results indicate high accuracy of the proposed method in predicting the concentration of polyphenols. The proposed method requires no tedious sample preparation and digestion steps, as compared with the national standard method, thereby greatly reducing the analysis time. Therefore, the method has strong potential and could be used reliably for the detection and quantification of polyphenols during the fermentation process of tea extract.

4. Conclusion
In the current work, a novel method for detecting the aroma of tea extract fermentation under ultrasound treatment is proposed. The study focused on the principle of using ultrasound treatment to improve the rate at which polyphenols are consumed during the fermentation process of tea extract. The proposed method respectively enhanced the consumption rate of polyphenol by 35.57%, 11.34% and 16.09% under the optimized 23 kHz of ultrasonic frequency, 24 min treatment time and 40 W/L ultrasonic power density, against the control fermentation experiment till the fifth day. Meanwhile, a novel approach based on NIR spectroscopy and a nano-optical sensor employing MOFs, PSA and PSN, monitored the polyphenols. The application of a comparative study of the prediction models to the spectral data revealed that the best results were obtained with the GA-PLS as compared to the ACO-PLS and CARS-PLS algorithm models. As a robust prediction model, GA-PLS was successful in capturing the relevant information as well as eliminating the uninformative variables related to the studied polyphenols. Finally, the correlation coefficient of prediction of 0.9988, and RMSEP values of 0.0264 mg/kg were obtained. The present work shows great potential in aroma analysis for tea extracts 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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Table 3
Comparison of the new method with GB method for the determination of tea polyphenols.

| Measured      | Si-GA-PLS | Si-CARS-PLS | Si-ACO-PLS |
|---------------|-----------|-------------|------------|
| GB/T 21,733  | 0.684     | 0.708       | 0.704      |
| 2008 Nanosensor-NIRS | 0.695 | 0.699       | 0.701      |
| 0.698       | 0.713       | 0.546   | 0.626      |
| 0.714       | 0.706       | 0.846   | 0.699      |
| 0.732       | 0.711       | 0.701   | 0.713      |
| 0.828       | 0.839       | 0.825   | 0.842      |
| 0.832       | 0.839       | 0.824   | 0.842      |
| 0.863       | 0.822       | 0.701   | 0.830      |
| 0.984       | 1.003       | 1.000   | 1.007      |
| 0.995       | 1.003       | 1.000   | 1.007      |
| 1.035       | 1.005       | 0.988   | 1.004      |
| 1.421       | 1.468       | 1.340   | 1.443      |
| 1.443       | 1.456       | 1.464   | 1.465      |
| 1.467       | 1.456       | 1.464   | 1.465      |
| 1.841       | 1.862       | 1.928   | 1.866      |
| 1.868       | 1.864       | 1.903   | 1.865      |
| 1.873       | 1.863       | 1.898   | 1.865      |
| 2.123       | 2.148       | 2.226   | 2.116      |
| 2.135       | 2.156       | 2.133   | 2.160      |
| 2.200       | 2.134       | 2.034   | 2.141      |

Fig. 7. Model Si-GA-PLS, Si-CARS-PLS and Si-ACO-PLS of predicting polyphenol content of tea extract fermentation broth under ultrasonic treatment.
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