Study the impact of ultra-sonication and pulsed electric field on the quality of wheat plantlet juice through FTIR and SERS

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A B S T R A C T
Pulsed electric field (PEF) and Ultrasound (US) are commonly used in food processing. We investigated the combined impact of pulsed electric field (PEF) and ultrasound (US) on the wheat plantlet juice. When compared with the individual treatments, the highest values of total phenolics, total flavonoids, chlorophyll, ORAC assay, and DPPH activities were obtained using the combined (US + PEF) methods. The US + PEF significantly decreased the peroxidase and polyphenol oxidase activities from 0.87 to 0.27 Abs min−1 and 0.031–0.016 Abs min−1. Also, the synergistic application significantly lowered the yeast and mold (3.92 to 2.11 log CFU/mL), E. coli/Coliform (1.95 to 0.96 log CFU/mL), and aerobics (4.41 to 2.01 log CFU/mL). Furthermore, Fourier Transform Infrared (FT-IR) and surface-enhanced Raman spectroscopy (SERS) was used to analyzing juice quality. Gold nanoparticles (AuNPs) were used as the SERS substrates, which provided stronger Raman peaks for the samples treated with US + PEF methods. The FT-IR analysis showed significant enhancement of the nutritional molecules. The enhanced quality of wheat plantlet juice combined with lower yeast and mold suggests the suitability of integrated methods for further research and applications.

1. Introduction

Recently, consumers’ demand is more oriented towards nutrient-rich foods, including sprouts, due to high phytochemicals and bioactive composition [1]. Health organizations also emphasized enhancing the daily intake of vegetables and fruits in their diet. Wheat plantlet corresponds to the newly sprouted first leaves of the wheat (Triticum aestivum) crop, widely used recently as a food supplement [2]. In wheat plantlets, the most active component is chlorophyll which inhibits carcinogens and is also a great source of antioxidants, minerals (Ca(II), Fe(II) and Mn (IV)), and vitamins (A, C, and E) [3]. Keeping in view the consumers’ preferences toward minimally processed and fresh juices, there is an extreme necessity to develop numerous innovative processing techniques [4]. Therefore, the food industry is trying to pay heed to develop new techniques for preserving healthy food such as juices for a longer time with negligible reduction in nutrient content.

The application of non-thermal food processing is emerging due to its non-interruptive nature with the sample. Among them, ultrasound (US), irradiation, pulsed electric field (PEF), cold plasma, ultraviolet, and high-pressure processing (HPP) are impressively appropriate as a substitute for thermal technologies [5–8]. With the combined impact of PEF and US, the nutritional value of food products can be improved, delivers higher microbial safety, and enhances the food’s nutrients without thermal deprivation [4,9]. Further, these novel processing techniques reduces processing time, economic, fewer energy inputs, efficiency, high throughput, constancy for reducing the activity of microorganisms, and environment friendly [10–12].

Recently, some innovative spectroscopic methods such as visible absorption, circular dichroism, infrared, fluorescence, Raman, linear dichroism, and nuclear magnetic resonance spectroscopies are extensively used for different foods to assess fruits and other agricultural products [13,14]. Surface-enhanced Raman spectroscopy (SERS) is a fast
spectroscopic tool, renowned for its sensitivity towards a wide variety of biologically active compounds [15]. In the current study, the impact of US and PEF and combined treatment on the bioactive compounds, enzyme activities, and microbial loads of wheat plantlet juice was analyzed. This work aimed to show the results of SERS to assess the impact of US, PEF, and combined treatment on wheat plantlet juice.

2. Materials and methods

2.1. Seeds selection, cultivation, and juice extraction

Wheat (Triticum Aestivum L.) variety (Inqilab-91) was obtained from Nowshehra Cereal Crop Research Institute (CCRI), Pakistan. The wheat plantlet was grown in the lab in Wushan campus, South China University of Technology, Guangzhou. Distilled water was used for seeds soaking for 24 h and then drained. Next, in a muslin cloth, seeds were covered and allowed for germination for 12 h. In the soil trays, the seeds were kept in sterilized and sealed glass bottles and kept at 4 °C till further examination.

2.2. Ultrasound (US) treatment

After obtaining the plantlet juice, the US treatment was performed. One 100 mL wheat plantlet juice was treated with US wave in an ultrasonic machine (SKYMEN JP-031S, Shenzhen, China) cleaner at a radiation 70%, frequency 40 kHz, temperature (30 ± 2 °C), processing time (20 min), and power 420 W. The water circulated at 0.5 L/min maintains the temperature of the cleaner. To avoid any possible obstruction of light, the US treatment was executed in the dark.

2.3. Pulsed electric field (PEF) treatments

By using a continuous PEF system, 100 mL juice of wheat plantlet was treated. Pumping of juice was performed through Longer Precision Pump (Y21515x, Hebei, China) at a pulse frequency of 1 kHz, pulse width of 80 μs, with an electric strength of 9 kV/cm, for 335 μs time at 30 ± 2 °C with a flow rate of 50 mL/min. For controlling the temperature, a thermometer was suspended in a water bath. A digital oscilloscope was employed to maintain the input voltage.

2.4. Combined treatment (US + PEF)

Simultaneous US + PEF was applied by treating the sample with the US and then followed by PEF. After completing all the treatments, the resultant solution was delivered through a muslin cloth, and juice was kept in sterilized and sealed glass bottles and kept at 4 °C till further examination.

2.5. Total phenolic contents (TPC) and total flavonoid contents (TFC)

TFC of the juice was tested by the following method proposed by Kim et al. [16]. A specimen of 500 μL was briefly combined with 1.25 mL double-distilled (dd) H2O and 75 μL NaNO2 (5%) solution. A solution of 150 μL of AlCl3·H2O (10%) was mixed to the above sample after passing 6 min. Subsequently, 0.5 mL NaOH (1 M) solution was injected at 5 min, and finally, the results against the blank were recorded at 510 nm.

Moreover, Slinkard and Singleton’s [17] proposed method was used for the estimation of TPC. 500 μL sample solution was combined with phenol reagent Folin-Ciocalteu (0.5 mL) and dd H2O (1 mL). Further, 2.5 mL Na2CO3 with 20% concentration was mixed before allowing the solution in a dark room for 20 min. Finally, the absorbance was accessed at 735 nm against a blank using a spectrophotometer (UV–visible-1810, Beijing, China).

2.6. Total antioxidant capacity (TAC) and DPPH scavenging activity

The DPPH activity in juice samples was ascertained by following the protocol described by Manzoor, Zeng, Rahaman, Siddeeg, Aadil, Ahmed, Li and Niu [9]. Estimation of TAC was done by Aadil, Zeng, Ali, Zeng, Farooq, Han, Khalid and Jabbar [18] proposed methodology. A precisely measured aliquot of sample juice was put in a flask (0.4 mL, 250 μg/mL in methanol), and 4 mL of reagent solution consisting of 0.6 M sulphuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate was introduced in the vial. Further, the solution was incubated for 90 min at 95 °C in a water bath. The blank solution was comprised of 0.4 mL methanol and 4 mL reagent solution. The mixture temperature was reduced to 25 °C, and then absorbance was calculated at 695 nm. Ascorbic acid (100–400 μg/ml) was exploited as a standard solution to draw a suitable calibration curve. The antioxidant property of the sample was recorded by comparing it with ascorbic acid. The percentage of DPPH was measured with the help of equation (1).

\[
\text{DPPH} \% = 1 - \frac{A \text{ sample} - A \text{ blank}}{A \text{ control} - A \text{ blank}} \times 100
\] (1)

2.7. Chlorophyll contents

The estimation of chlorophyll contents was done according to Zhao et al. [19] protocol. In detail, acetone (80% v/v; 3 mL) was mixed to control and treated wheat plantlet juice (3 mL) followed by filtering the solution (thrice) by using a Whatman filter paper (125 mm Ø 100 circles; wet strengthened circles Springfield Mill, England). At room temperature, the filtrate absorbance was estimated at 663 nm and 645 nm. The total chlorophyll values were calculated by employing the following formulas:

\[
\text{Chlorophyll } a = (11.85 \times A664) - (1.54 \times A647)
\] (2)

\[
\text{Chlorophyll } b = (21.03 \times A664) - (5.43 \times A647)
\] (3)

\[
\text{Total chlorophyll} = (\text{chlorophyll } a) - (\text{chlorophyll } b)
\] (4)

2.8. Peroxidase (POD) and polyphenol oxidase (PPO) activity

To analyze the activity of PPO and POD in wheat plantlet juice (Cano, Hernandez, & DeAncos, 1997) proposed method was used with some changes. Centrifugation of Juice samples was done for 5 min at 5,000 × g and poured out supernatant. To measure the POD, the supernatant was diluted 100-times by using distilled water and transferred 1 mL of this diluted supernatant to a tube with 0.32 mL potassium phosphate buffer (pH 6, 0.1 M) and 5% (w/v) pyrogallol 0.16 mL of at 25 °C. 0.16 mL of hydrogen peroxide (0.5% w/w) was added to imitate the reaction, and for 5 min, then an increase in absorbance at 485 nm was examined. Catechol 1 mL (0.07 M) in phosphate sodium buffer (pH 6.5, 0.05 M) was added in diluted supernatant for the evaluation of POD assay and monitored the enhancement in absorbance in 10 min at 420 nm over at room temperature (25 °C). The POD/PPO activity (Abs min⁻¹) was calculated by the slope of the early linear portion of the reaction curve.

2.9. Microbiological analysis

Microbial loads determination was carried out by the procedure described in FDA’s Bacteriological Analytical Manual [20]. The pour plate method with nutrient agar medium was used to performed total plate counts, while the PDA medium was used for yeast and mold counts. The results were described as log CFU/mL.

2.10. FTIR spectroscopy

The FTIR spectra provide structural information based on functional
groups and confirm the compositional changes in a sample. The IR spectra were obtained using the FTIR spectrometer (Vector 33, Ettlingen, Germany). The IR spectrum ranged from 4000 to 400 cm$^{-1}$ with 4 cm$^{-1}$ resolution. The FTIR measured the functional groups of the wheatgrass juice.

2.11. Determination of juice quality through SERS

2.11.1. Preparation of AuNPs

Zheng and He [21] reported the mechanism of surface-enhanced Raman spectroscopy (SERS) as an emerging and promising technique for the chemical analysis of food. They reported the use of metallic nanosubstrates for the better sensitivity and capacity of conventional Raman spectroscopy. More recently Zhao, Li and Xu [22] reported surface-enhanced Raman scattering (SERS) as a powerful testing technology due to its sensitivity, rapidity, and non-destructive damage to the sample. Therefore we decided to use AuNPs for the SERS application. AuNPs are easy to prepare and have proved promising in enhancing Raman signal up to many folds by chemical and physical processes. Chemical enrichment is the enhancement of signal due to charge transfer between nanosubstrates and analyses (enhances up to 10$^2$ times) whereas, the physical effect is due to option properties of nanoparticles which contributes to improving signals up to 10$^{12}$ folds [23,24].

Synthesis of AuNPs was conducted by employing the protocol adopted by Hussain, Pu, Hu and Sun [24]. Briefly, one milliliter of HAuCl$_4$·4H$_2$O (5 g/L solutions) was first poured in a flask (150 vol) containing 60 mL of ultrapure water with continuous shaking at 900 rpm with a digital shaker (MS 3, IKA Inc., Staufen im Breisgau, Germany). Further, boiled the solution at 120 °C for 1 min and then introduced 700 μL trisodium citrate (1% concentration) and then boiled for 5 min. The color change of solution (colorless to purplish-red) confirmed the synthesis of AuNPs. For the SERS study, the US, PEF, or US + PEF treated sample (200 μL) was mixed with 100 μL AuNPs followed by mixing for 1 min, and finally, Raman analysis was performed.

To determine the successful preparation of AuNPs, TEM images were acquired and depicted in Fig. 1A. Images present circular and uniform nanoparticles of about 33 nm in diameter. Such images were also observed by [13]. Furthermore, to determine the average size and aggregation in AuNPs, the dynamic light scattering (DLS) technique was applied. The results showed no aggregation in the substrate with an average diameter of about 33 nm (Fig. 1B). Moreover, results from UV–Vis spectra for nanosubstrates showed a peak centered at 526 nm, as shown in Fig. 1C. The UV–Vis maximum absorbance peak was useful for the calculation of the average diameter of nanoparticles by using the following formula:

$$d = 2.99\lambda_{\text{max}} - 1539$$

(5)

where $d$ (nm) is the diameter of the substrate and $\lambda_{\text{max}}$ (nm) is the absorption peak wavelength.

The calculations also showed an average diameter of about 33.72 nm in diameter, similar to other obtained results.

2.11.2. Characterization of AuNPs

The morphology of the AuNPs was tested using TEM images, DLS tests, and UV–Vis absorption spectrophotometry. TEM images were acquired using a JEM-2100F transmission electron microscopy (JEM-2100F, JEOL Ltd., Tokyo, Japan). For which, 3 mL freshly synthesized

Fig. 1. Shows the (A) TEM images, (B) DLS results, (C) UV–Vis spectra for AuNPs.
nanoparticles were first centrifuged at 4000 rpm for 10 min, followed by removal and addition of 3 mL ultrapure water. This step was performed twice, and the obtained solid was diluted and subjected to ultrasonic for 25 min. Finally, the solutions were dropped on ultra-thin carbon film (T11032, Beijing Xinxing Brim Technology Co., Ltd., Beijing, China) and allowed to dry at ambient temperature.

Moreover, the hydrodynamic diameter of SERS-substrate was analyzed by employing DLS with a Zetasizer system (Malvern Zetasizer Nano ZS, Malvern Instruments, and Worcestershire, UK). Furthermore, the UV–Vis absorbance of AuNPs was determined with a UV-1800 spectrophotometer (Shimadzu Co., Ltd., Kyoto, Japan) by setting the absorbance ranging from 350 to 500 nm. For DLS analysis and UV–Vis absorption, the freshly prepared substrate was dissolved in water by 1:1 vol ratio, and then experiments were done.

2.11.3. Acquisition of SERS spectra

To check the effect of US, PEF, and their combined impact on wheatgrass juice, a confocal Raman tool equipped with a 633 nm laser (Horiba France SAS, Villeneuve d’Ascq, France) with 50 mW laser was used. The system was also furnished with a high steady confocal microscope (USA, PA, BX41, Olympus Co.), a grating of 600 grooves per mm, confocal adjusted optics between the microscope and the spectrograph, an 800 mm focal length achromatic flat field monochromator, and had a cooled charge-coupled device detector. About 1 mm of the diameter of the capillary tube for liquid samples was employed. Raman spectra were collected from 400 to 1700 cm$^{-1}$ via a 10X objective with laser power as 100%, the exposure time as 10 s, with two sets of accumulation adjustment. The obtained spectra were further processed for denoising, despising, and baseline correction as necessary preprocessing (spectral) steps as described by [25].

2.12. Statistical analysis

All trials were accomplished in triplicate. SPSS version 24 (IBM SPSS Statistics, Armonk, NY) was exploited for statistical assessment of experimental data. In this research, the p < 0.05 value was considered statistically significant.

3. Results and discussion

3.1. Effect on TPC and TFC

Table 1 shows a significant (p < 0.05) rise in TPC and TFC with the US, PEF, and US + PEF than untreated wheatgrass juice sample. TFC values increased from 178.34 ± 0.11 µg CE/g (untreated) to 193.67 ± 0.11 with the US, 188.17 ± 0.16 in PEF, and 203.42 ± 0.18 µg CE/g in US + PEF applied juice. Similarly, some studies have shown a significant rise in TFC in sonicated pear juice [26] and a rise in flavanone in HIPEF treated strawberry juice [27]. As compared to untreated juice (305.23 ± 0.11 µg GAE/g), TPC increased with US (315.32 ± 0.14 µg GAE/g), PEF (321.56 ± 0.08 µg GAE/g), and US-PEF (331.45 ± 0.17 µg GAE/g) treated wheatgrass juice. Likewise, PEF application with permeabilization also facilitates the extraction of the intracellular components, results in increasing yield, extraction performance, and aids in intracellular metabolites extraction [28]. In PEF treatment, EF intensity higher than the cell membrane capacity, rupturing the cell membrane, which raises the dissolution rate [29]. The rise of TPC and TFC ultimately increases antioxidant attributes beneficial for health [29]. During the US, the increase in TPC might be due to the discharge of the bound form of phenolics through the cell membranes ruptured due to the cavitation process [30,31]. A similar increment in TPC was also recorded in PEF applied apricot juice [32] and the US-treated sugarcane juice [6]. The increment in TPC and TFC poses beneficial health impacts on consumers due to the rise in antioxidant potential [33]. In the current study, the combined effect of US + PEF also significantly increased TFC and TPC in the tested juice. Our findings are in line with a previous evaluation conducted by coupling HPP + US application on apple juice [34] and US and PEF on spinach juice [7]. Our outcomes proposed that the combination of multiple approaches can be the best option to get the best outcomes regarding health-promoting phytochemicals.

3.2. Effect on ORAC and DPPH activity

To determine the antioxidant ability of wheatgrass juice, ORAC and DPPH assays were considered reliable methods. The assessment of ORAC and DPPH assays showed high antioxidants activity of juice (Table 1). The wheatgrass juice DPPH value against untreated, US, PEF, and US + PEF were 1.63 ± 0.03, 1.69 ± 0.06, 1.71 ± 0.02, and 1.74 ± 0.07, respectively. At the same time, ORAC values were 5.12 ± 0.05, 5.18 ± 0.03, 5.19 ± 0.08, and 5.24 ± 0.06 against untreated, US, PEF, and US + PEF, respectively. In terms of antioxidants, the US + PEF significantly increased both the ORAC and DPPH assays for the wheatgrass juice [35]. US + PEF application on wheatgrass juice showed the highest DPPH radical scavenging and ORAC assays than the US and PEF methods. During US treatment, the increment was due to phenolic molecules, which rise during the cavitation and cause the hydroxylation of flavanoids, creating progressive antioxidant contents [36]. Moreover, PEF under the particular electric field strength induces structural changes in protein, including the polarization in a molecule, molecular mass, and quaternary structure, rise or decline in DDPH inhibition [37]. Da Silva et al. [38] stated that the total antioxidant capacity (TAC) and DPPH radical scavenging properties of fruits and vegetables depend on phytochemicals, such as flavonoids, vitamins, and carotenoids or phenols. Nevertheless, PEF treatment was significant for radical development related to the high-energy input, which was monitored to enhance the antioxidative capacity [39]. Likewise, previously testified higher scavenging DPPH level in sonicated blackberry juice than untreated samples [40]. The significant increase in ORAC and DPPH activity after combined treatment confirmed the synergistic effects of both US and PEF applications on the wheatgrass juice. Our results are in agreement with the integrated research conducted by Faisal Manzoor, Ahmed, Ahmad, Karrar, Rebman, Muhammad Afdil, Al-Farga, Wabed Iqbal, Rahaman and Zeng [7]. A significant increase in TAC and DPPH after the joint application of US and PEF on spinach juice was evaluated due to the

| Parameters | Untreated | US Treatment | US-PEF Treatment | US + PEF Treatment |
|------------|-----------|--------------|------------------|--------------------|
| TPC (µg GAE/g) | 305.23 ± 0.11$^c$ | 315.32 ± 0.14$^{bc}$ | 321.56 ± 0.08, 5.35 | 331.45 ± 0.17$^a$, 8.59 |
| TFC (µg CE/g) | 178.34 ± 0.09$^d$ | 193.67 ± 0.11$^{bc}$ | 188.17 ± 0.16, 5.51 | 203.42 ± 0.18$^a$, 14.06 |
| DPPH (TE mmol L$^{-1}$) | 1.63 ± 0.03$^d$ | 1.69 ± 0.06$^{de}$ | 1.71 ± 0.02, 4.90 | 1.74 ± 0.07$^a$, 8.58 |
| ORAC (TE mmol L$^{-1}$) | 5.12 ± 0.05$^d$ | 5.18 ± 0.03$^{de}$ | 5.19 ± 0.08, 1.36 | 5.24 ± 0.06$^a$, 2.34 |
| Chlorophyll (mg/100 mL$^{-1}$) | 1.74 ± 0.04$^{bc}$ | 1.79 ± 0.06$^{bc}$ | 1.81 ± 0.03$^b$, 4.02 | 1.92 ± 0.05$^a$, 12.06 |

US-PEF: Combined treatment of ultrasound and pulsed electric field; RC: Relative change. Values with different superscript letters within the same row are significantly different (P < 0.05) from each other. All values are indicated as mean ± standard deviation.
3.3. Impact on chlorophyll

The chlorophyll concentration of wheatgrass juice after PEF, US, and US + PEF treatments is displayed in Table 1. Findings presents significant \((p < 0.05)\) enhanced in chlorophyll content from 1.74 (untreated) to 1.79, 1.81, and 1.92 mg/100 mL in the US, PEF, and US-PEF, respectively. Also, the PEF and US application could deteriorate in chlorophyll degradation from the juice. The outcome was also inconsistent with the former studies; for instance, PEF application showed significant stability in spinach puree pigment due to the microbial and enzymatic destructions responsible for the degradation of chlorophyll [41]. In the current work, the stability of pigments might be due to the changes in the microenvironment of the sample, as consequences of PEF application. Moreover, the crosslinking bonds with other pigments (chlorophyll) result in the formation of chlorophyll self-aggregation, which enhances the permanence of chlorophyll compounds. Similarly, the US-treated juice sample showed the retention of chlorophyll levels, while spinach juice treated with high hydrostatic pressure (HHP) also delivered similar findings. The results were obtained due to removing the chlorophyll-protein complexes photosystem II [42]. Likewise, enhancement in chlorophyll extraction was obtained with US treatment, consequents in broadening of cell wall pores due to swelling and hydration process [43].

Meanwhile, US and PEF integration also caused a significant enhancement in total chlorophyll levels compared to the untreated samples. A similar study was also reported on spinach juice treated with PEF and US simultaneously [7]. The possible cause for cell disruption happened during US and PEF treatment outcomes in the discharge of chlorophyll from plantlets.

3.4. Effect on enzymatic activities

PPO and POD are generally reduced oxidative stress present in plant tissue, which are spoilage enzymes responsible for color variations, specifically concerning browning; they can differ instability depending on the sources [44]. In the current study, US, PEF, and US + PEF treatments significantly \((p < 0.05)\) inactivated both enzymes, as presented in Table 2. The PPO activity reduced considerably in all samples than in the untreated sample. The US and PEF reduce the PPO activity of wheat plantlet juice whereas, the US + PEF application achieved the lowest reduction value. The enzyme inactivation process with PEF is not understood correctly yet but, it is anticipated that the non-thermal approach may alter the conformational state of the enzymes. Also, Zhao, Yang, Lu, Tang and Zhang [45] noticed the loss and decline of lysozymes with the PEF technique. Similarly, loss of secondary \(\alpha\)-helix structure in papain and lysozyme was observed after treatment with the HIPEF method [46]. Moreover, PEF alters the protein inactivation conformation and polarizes the protein molecule, which declines the PPO and POD enzymes [41]. Previously, Sánchez-Vega, Elez-Martínez and Martín-Belloso [47] described a considerable decline in PPO and POD in PEF applied broccoli juice and HHP treated watermelon juice [48]. During US application, magnetostriuctive impressions are generated, producing cavitation, resulting in conformational changes in enzymes [49]. Kubo, Curet, Boilireaux and Augusto [50] recorded variation in PPO and POD values in fruit juice after US application compared to untreated samples. Additionally, the synergistic impact of US + PEF resulted in the maximum decline for PPO and POD in wheatgrass juice. The inactivation of the enzymes was also reported through UV and PEF application [51] and with a coupling of PEF with US [52]. The decline in browning is a positive aspect of the PPO and POD enzyme inactivation [53]. The retaining of enzymatic activity with raw juices is beneficial, giving great health benefits [54].

3.5. Impact on microbial loads

The outcomes for samples treated with coupled methods are depicted in Table 2. Significant reduction in yeast and mold (Y&M), E. coli/Coliform, and aerobic were recorded with combined US and PEF application compared to control samples. Similarly, a decline in the E. coli/Coliform, Y&M, and aerobics were noticed during the US, PEF, and US-PEF treatments. In contrast, untreated Y&M, E. coli/Coliform, and aerobics delivered 3.83, 1.90, and 4.23 log CFU/mL, respectively. However, the highest decline was recorded with simultaneous application of US and PEF. Likewise, a reduction in microbial activity in juices from pear and Kasturi lime was noticed with the sonicated method [26]. The decline in microbial loads results in the cavitation process due to chemical and physical breakage. An increase in the localized heat and free radicals is produced during cavitation, which is involved in reducing the microbial load [55]. Joyce, Phull, Lorimer and Mason [56] experimentally proved that ultrasound can inactivate bacteria and deagglomerate bacterial clusters through some physical, mechanical, and chemical effects arising from acoustic cavitation. According to Xie, Li, Hou, Yang, Li, Li and Du [57] TEM-analysis prove that US treatment significantly changes the internal organelles of bacteria thereby kills them through production of reactive oxygen species (ROS) observed through laser scanning confocal microscopy and flow cytometry; hence ultrasound is an experimentally proven, efficient bactericidal technique.

Huang et al. [58] confirmed the potential of PEF treatment to initiate changes in the cell structure by rupturing its membrane, the vital endogenous enzymes linked with the cellular processes, alter gene expression, and interrupt genetic material. Earlier, Kayalvizhi et al. [59] also noticed a decline in microbial load in sugar cane juice when applied the PEF technique. Grahl and Mark [60] reported the lethal effects of PEF on suspensions of various bacteria, yeast, and spores in buffer solutions and liquid foodstuffs. For each microorganism cell type, specific critical electric field strength and a specific critical treatment time were determined. Above these critical values, the fractions of surviving microorganism cells were reduced drastically. In addition to the inactivation of microorganisms, the effect of PEF on food nutrients such as whey proteins, enzymes, and vitamins was studied. The degree of destruction of these food nutrients by the PEF was very low or negligible.

Zhang et al. [61] also determined a reduction in microbiological activity due to electrosorption and electroproportion when treated with PEF;

| Parameters | Untreated Treatment | US Treatment | RC% | PEF Treatment | US + PEF Treatment | RC% |
|------------|---------------------|--------------|-----|---------------|-------------------|-----|
| POD (Abs min \(^{-1}\)) | 0.87 ± 0.07\(^a\) | 0.65 ± 0.08\(^b\) | –25.28 | 0.56 ± 0.04\(^{bc}\) | –35.63 | 0.27 ± 0.06\(^d\) | –68.96 |
| PPO (Abs min \(^{-1}\)) | 0.031 ± 0.001\(^a\) | 0.021 ± 0.001\(^b\) | –32.25 | 0.019 ± 0.002\(^b\) | –38.70 | 0.016 ± 0.001\(^c\) | –48.38 |
| E. Coli/coliform (log CFU/mL) | 1.95 ± 0.03\(^a\) | 1.53 ± 0.04\(^b\) | –21.53 | 1.58 ± 0.05\(^a\) | –18.87 | 0.96 ± 0.02\(^e\) | –50.76 |
| Yeast & Mold (log CFU/mL) | 11.13 | 3.11 ± 0.07\(^a\) | –60.17 | 2.11 ± 0.03\(^e\) | –46.17 | |
| Aerobics (log CFU/mL) | 4.41 ± 0.03\(^a\) | 3.53 ± 0.05\(^b\) | –19.95 | 2.42 ± 0.02\(^c\) | –45.12 | 2.01 ± 0.04\(^d\) | –54.42 |

US-PEF; Combined treatment of ultrasound and pulsed electric field, RC; Relative change. Values with different superscript letters within the same row are significantly different \((p < 0.05)\) from each other. All values are indicated as mean ± standard deviation.
the US + PEF resulted in microbial reduction than the individual US, PEF application, and untreated samples. Likewise, Manzoor et al. [4] also ascertained a significant decrease in microbial load in spinach juice by PEF and US application.

3.6. FTIR analysis of US, PEF, and US + PEF treated samples

Spectral data for wheat plantlets treated with US, PEF, and US + PEF is depicted in Fig. 2. An identical spectrum with a similar band position was achieved for all the juice samples. The transmittance percentage of identical C = O groups showed dissimilarities in all the treatments. The broad peak (3000–3600 cm\(^{-1}\)) in the IR spectra region presented the O–H stretching vibrational area in the carboxylic group. The results highlight the tenancy of R-COOH due to the stretching of O–H moiety at 3000 cm\(^{-1}\) and the faint disappearance of C–H stretching in broadband spectra [62]. The deformation vibrations of C–H at 1450–1650 cm\(^{-1}\) an absorbance peak at 3400–2800 cm\(^{-1}\) showed the existence of an alkane group. Absorption bands in –CH\(_3\) groups showed the asymmetric and symmetric stretching at 2800–2950 cm\(^{-1}\). According to the study of Szalontai, Kóta, Nonaka and Murata [63], the narrow and sharp band was also ascertained at 1750 cm\(^{-1}\), corresponds to stretching of the C = O and ester group. In this study, stretching vibrations of the C-O group disappeared at 1300–1650 cm\(^{-1}\), depicting the removal of the ketonic group. A weak –NH spectra at 1400 cm\(^{-1}\), with a strong peak, was also noticed at 1500 cm\(^{-1}\) in aliphatic secondary amines. The noteworthy differences of % transmittance in the treated can be achieved from carboxylic acids, amine, and ester. The increase of % transmittance in the ester group might be due to the ejection of polarity and color formation. Outcomes of the treatment of US, US + PEF, and PEF presented the prominent differences in the % transmittance of C = O compounds. Comparable FT-IR spectral data analysis was noticed in the PEF and US-treated extract of almonds [9] and spinach juice [4].

3.7. SERS analysis of US, PEF, and US + PEF treated samples

To check the influence of PEF, US, and US + PEF on juice composition, we employed SERS based on AuNPs as a sensitive and non-destructive tool. SERS is an improvement in normal Raman spectroscopy, in which weak Raman scattering in a later method is enhanced to many folds by using nanoparticles for sensitive detection applications [13,25,64]. The Raman spectra for the sample without any treatment showed a weak spectrum, as shown in Fig. 3. Whereas PEF treated juice delivered more substantial peaks than US samples. Furthermore, US + PEF for the sample treatment significantly enhanced the Raman intensity compared to single methods applications, as depicted in Fig. 3. The variation in Raman intensities may be due to differences in compositional concentrations in untreated, ultrasonicated, pulse electric treatment and a synergistic effect of ultra-sonication and pulse electric field method for the treatment of wheat juice. Raman assessment of the wheat juice showed some firm peaks at 733 cm\(^{-1}\) (assigned to carbohydrates), 964 cm\(^{-1}\) (due to cutin and polysaccharides), 1330 cm\(^{-1}\) (correspondent to aliphatic compounds), 1458 cm\(^{-1}\) (due to aliphatic compounds, cutin, and waxes) and 1617 cm\(^{-1}\) (shows aliphatic and aromatic compounds). Also, weak or medium peaks were detected at 627 cm\(^{-1}\) and 1552 cm\(^{-1}\) positions, corresponding to the phenyl ring of flavonoids and Amide II, respectively. These Raman responsive peaks were due to different deformation, stretching, bendings, or scissoring of different bonds in the samples. Details of peaks and their assignment are presented in Table 3. The peak assignments were also reported by various researchers in plant-based products [65–67]. Earlier, Malekfar, Nikbakht, Abbasian, Sadeghi, and Mozaffari [68] determined the tomato juice quality by SERS. SERS spectra demonstrated that SERS has a privilege in the assessment of proteins and carbohydrates as an efficient, precise, and rapid technique. Some useful resources on SERS theory, mechanism, and applications have been reported [14, 15, 69]. Briefly, to achieve the effective SERS enhancement mechanism, there must be critical resonance between the metal (substrate) and the applied laser with selected wavelengths (532 nm, 633 nm, and 780 nm). The most commonly used metals for SERS are silver and AuNPs. The SERS effect can be achieved when an analyte (food sample) is adsorbed onto or near a prepared metal surface of nanomaterial (AuNPs). The Raman excitation laser produces surface plasmons (coherent electron oscillations), on the surface of the metal.

![Fig. 2. FT-IR analysis of untreated, PEF, US, and US + PEF treated wheat plantlet juice samples.](image)

![Fig. 3. Shows comparative Raman spectra for (A) untreated, (B) PEF treated (C) US treated, and (D) combined US + PEF on wheat plantlet juice.](image)

| Raman peak (cm\(^{-1}\)) | Assignment | Reference |
|--------------------------|------------|----------|
| 627                      | Aromatic C-H out of plane deformation and C-H deformation in phenyl rings of flavonoids | [65] |
| 733                      | The band resulting from C-C and C-O stretching vibrations of carbohydrates and bending of (CH\(_2\)) rocking of cutin waxes | [64,65,68] |
| 964                      | Stretching of (C-O) of cutin and polysaccharides | [66,70] |
| 1330                     | CH\(_2\) bending, aliphatic compounds | [65] |
| 1458                     | (CH\(_2\)) scissoring and represents aliphatic compounds, cutin, and waxes | [67] |
| 1552                     | Amide II: N–H deformation Contribution from C-N | [71,72] |
| 1617                     | Stretching of (C – C\(_2\)), indicating the existence of both aliphatic and aromatic C = C double bonds | [73,74] |

Table 3: Raman peaks observed from wheat juice and their assignments.
These surface plasmons interact (transfer to) with the analyte to greatly enhance the Raman emission recorded by the Raman Spectrometer. Our experimental results support these observations therefore we conclude SERS as an efficient testing method for our food samples.

4. Conclusions

We investigated the impact of US, PEF, and US + PEF on some physicochemical and biological attributes of wheat plantlet juice. Experimental results showed a significant increment in TPC, TFC, ORAC, DPPH, and chlorophyll. Also, a significant microbial reduction and lower enzyme activities were observed. Two spectroscopic tools, SERS based on AuNPs and FTIR, were used as the experimental evidence for the best-combined treatment (US + PEF). Stronger Raman peaks due to the efficient SERS effect were observed for the wheat plantlet juice treated with the US + PEF. The FT-IR spectra for all the samples showed a rise in the concentrations of the nutrition (flavonoids, chlorophyll, ORAC assay, and DPPH activities). Therefore our investigation revealed that samples treated with multiple non-thermal methods US + PEF are safer with higher nutrients.

CRediT authorship contribution statement

Zahoor Ahmed: Conceptualization, Methodology, Formal analysis. Muhammad Faisal Manzoor: Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing. Abid Hussain: Formal analysis, Writing - original draft, Writing - review & editing, Muddasar Hanif: Writing - review & editing, Zia-ud-Din: Data curation, Software. Xin-An Zeng: Supervision, Funding acquisition.

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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