



OPEN A peak comparison index approach for robust microplastic analysis across environmental matrices: validation using meat products

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Microplastic contamination in food products raises significant concerns regarding human health and environmental safety, necessitating accurate detection methodologies for complex organic matrices. This study presents an effective framework for microplastic detection in meat by systematically evaluating four chemical digestion methods: hydrogen peroxide (H₂O₂), hydrochloric acid (HCl), sodium hydroxide (NaOH), and Fenton's reagent. Among these, HCl and NaOH demonstrated the highest organic matter removal efficiencies while preserving polymer integrity across polyethylene (PE), polystyrene (PS), and polyvinyl chloride (PVC) samples. A novel peak comparison index (PCI) quantitatively assesses spectral alterations, identifying NaOH as the least disruptive digestion method. Furthermore, the systematic comparison of digestion-induced polymer modifications enhances the standardization of microplastic analysis, facilitating methodological harmonization across food systems and broader environmental matrices. These findings establish a robust analytical approach for improving microplastic detection accuracy, strengthening risk assessments, and supporting contamination mitigation strategies within food supply chains.

Keywords Microplastics, Meat products, Fourier transform infrared spectroscopy, Digestion, Peak comparison index

Microplastics (MPs) have emerged as pervasive environmental pollutants, raising global concerns due to their widespread presence and potential impacts on ecosystems and human health.

In the livestock industry, MPs have recently been identified in various biological samples, such as feces and digestive systems of sheep^{1,2}, cow milk³, and cow blood and meat⁴. The primary sources of this exposure are the use of plastic materials on farms, such as silo covers and hay bale wrappings⁵. Additional sources include airborne microplastics, food production equipment, packaging, and supplementary ingredients^{6,7}.

Analyzing microplastics in biological samples is challenging due to the lack of standardized protocols for organic-rich materials⁸, necessitating methods to remove organic residues without altering microplastic properties⁹. Common approaches include oxidative, acidic, alkaline, and enzymatic digestion, with H₂O₂ and Fenton's reagent often used for oxidative processes⁹, while acidic or alkaline solutions like HCl, HNO₃, NaOH, and KOH are also applied, respectively^{10,11}. Enzymatic digestion is less common due to higher costs and slower processing, with proteinase-K being a typical example¹².

Understanding MP contamination in terrestrial meats and human exposure remains limited¹³. While MP accumulation in marine food webs is well-documented, studies on land-based proteins like raw meats or meat products are scarce (Table S1)^{7,14}. Milne et al.⁷ estimate that U.S. adults are exposed to ~ 11,000 MPs annually via these proteins, with maximum exposure reaching 3.8 million MPs. Research on MPs' health impacts is nascent, but exposure may cause chronic inflammation, neoplasia, and release toxic substances or adsorb pollutants like heavy metals, antibiotic resistance genes (ARGs), polycyclic aromatic hydrocarbons (PAHs), and pathogens^{15–17}. Further research is needed to identify contamination sources and mitigate risks⁷.

This study introduces the peak comparison index (PCI), a novel metric for quantifying alterations in FTIR peaks, to validate efficient methods for organic matter removal from microplastics. The aim is to develop techniques that swiftly eliminate organic residues, including meat and meat products, while preserving the integrity of microplastics and assessing chemical changes in their FTIR spectra.

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Materials and methods

Digestion solutions

To investigate the effects of various digestion solutions at different temperatures and digestion durations, and based on their frequent use in previous studies, the selected solutions were 30% H₂O₂, 37% (v/v) HCl, 1 M NaOH, and Fenton's reagent (30% H₂O₂ + 0.05 M FeSO₄·7 H₂O). The Fe (II) solution was prepared using the NOAA (National Oceanic and Atmospheric Administration) procedure¹⁸. All materials were purchased from Merck Millipore (Darmstadt, Germany).

Plastic samples

Plastic samples were collected from various refineries to evaluate digestion protocols. The samples included PE (90% HDPE and 10% LDPE) from PackPolymer (Tehran, Iran), PVC from Alvand Petrochemical Company (Shazand, Iran), and PS from Tabriz Petrochemical Company (Tabriz, Iran). Granules were in the size range of 25 μm to 45 μm using stainless-steel sieves. After immersion in digestion solutions for the specified duration and temperature, each sample was washed three times with ultrapure water and dried in a vacuum oven at 70 °C for 2 h¹⁹.

Digestion of organic matter

Fresh beef samples (rump cut, uniform fat content) were cut into ~1 g pieces, placed in prewashed tubes, and submerged in digestion solutions. For Fenton's reagent, depending on the protocol, ice-bath controlled temperatures exceeding 40–60 °C. Samples were weighed initially, incubated at 70 °C (instead of 105 °C) for 2 h to preserve microplastic integrity in accordance with PMMA's (Polymethyl Methacrylate) continuous operating temperature (COT) of 70°C²⁰, and then weighed again to obtain the final weight. The total mass loss (%Δm) directly reflected the organic material loss in samples containing microplastics:

$$\% \Delta m = \left(\frac{m_{\text{initial}} - m_{\text{final}}}{m_{\text{initial}}} \right) \times 100 \quad (1)$$

Where m_{initial} (g) is the initial mass and m_{final} (g) is the final mass.

Experimental design and procedure

This study first evaluated the efficacy of 30% H₂O₂, 37% HCl, 1 M NaOH, and Fenton's reagent for organic removal using a full factorial design (FFD). The experiments were conducted at 40 °C and 60 °C over 1, 2, 4, 6, and 12 h durations. A total of 10 trials were executed for each reagent in every iteration (a total of 120 runs for 3 replicates). The optimal conditions for each reagent at both temperatures and durations were subsequently determined, resulting in six distinct scenarios. The second phase tested these scenarios on PE, PVC, and PS polymers.

FTIR, ATR-FTIR, and PCI

The functional groups of the polymers were analyzed using a Frontier FTIR spectrometer (PerkinElmer, Waltham, USA). The spectral range examined was from 4000 cm⁻¹ to 400 cm⁻¹, utilizing 32 scans at a resolution of 1 cm⁻¹. In the FTIR analysis, the ratio of polymer to potassium bromide (KBr) and the pressure applied during tablet preparation were consistently maintained across all samples.

The peak comparison index (PCI) was defined to assess the intensity of each digestion method's effect. The PCI is calculated as the ratio of the change in transmittance of the IR spectrum for significant peaks of each polymer (see Table S2) after exposure to the respective scenarios, relative to the transmittance of the IR spectrum of the virgin sample. Equations 2 and 3 are used to calculate the PCI.

$$PCI_{p_i} \% = \left(\frac{T_{pSc_i} \% - T_{pV_i} \%}{T_{pV_i} \%} \right) \times 100 \quad (2)$$

$$PCISc_i \% = \frac{\sum_i^n |PCI_{p_i} \%|}{n} \quad (3)$$

Where PCI_{p_i} represents the peak comparison index for the i th peak, T_{pSc_i} denotes the IR transmittance of the i th peak for a selected polymer following the implementation of the specified scenario, T_{pV_i} refers to the IR transmittance of the i th peak for the virgin polymer, and $PCISc_i$ indicates the calculated peak comparison index for each polymer across various digestion protocols.

A lower absolute value of PCI indicates a reduced average change from each scenario compared to the untouched sample, suggesting that the sample has undergone less alteration. The benefit of employing the PCI over a comprehensive comparison of all IR spectra lies in its greater emphasis on functional groups. A schematic of the full experimental procedure is presented in Fig. 1.

QA and QC

Quality assurance (QA) and quality control (QC) are essential for reliable microplastics research. Effective QA/QC practices include instrument calibration, method validation, contamination prevention, and clean-air conditions²¹. Blank samples detect contamination, replicates assess variability, and certified reference materials ensure accuracy. Cleaning lab surfaces with ethanol further reduces contamination risks²². Implementing these measures enhances data reliability, advances environmental microplastics research, and supports regulatory strategies^{20,23}.

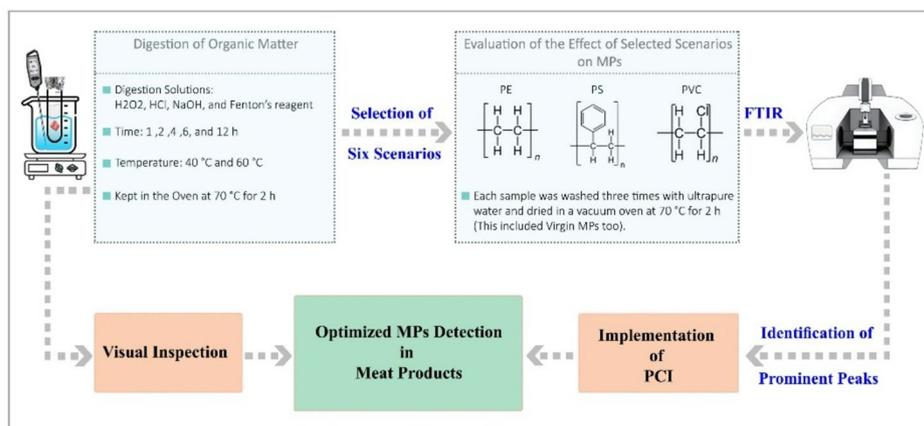


Fig. 1. Schematic procedure for selecting optimal practices in MPs sample processing.

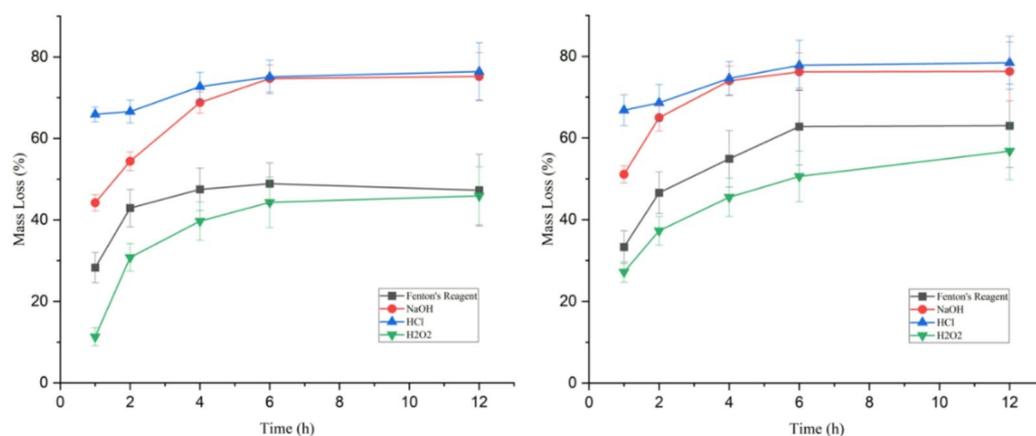


Fig. 2. Efficacy of chemical digestion in reducing the mass of fresh beef samples at (a) 40 °C, and (b) 60 °C.

Results

Efficacy of chemical digestion in reducing organic matter content

According to Fig. 2, HCl achieved the highest reduction in sample mass through organic matter loss, followed closely by NaOH. At 60 °C, HCl reduced the meat's mass by 77.8% and 78.4% after 6 and 12 h, respectively, while NaOH reduced 76.2% and 76.3% in the same periods. At 40 °C, the mass reduction rates for HCl and NaOH after 6 h were 75.1% and 74.7%, respectively. For H₂O₂ and Fenton's reagent, as shown in Fig. 2, the maximum reduction at 40 °C was 45.9% and 47.3%, respectively. At 60 °C and 12 h, H₂O₂ achieved a maximum reduction of 56.8%, while Fenton's reagent achieved 62.8% and 63.0% reduction after 6 and 12 h, respectively.

According to Fig. 3a, H₂O₂ bleached the organic matter (meat), while the Fenton process caused slight yellowing due to iron deposition (Fig. 3b). Both treatments had minimal impact on the sample. NaOH dissolved the meat gradually, producing a transparent pink liquid (Fig. 3c). HCl dissolved the meat faster than NaOH in the first 4 h, darkening the sample upon dissolution (Fig. 3d). NaOH-treated samples appeared visually superior for microscopic inspection.

Effect of chemical digestion on MPs

To assess the effects of chemical digestion, the prominent peaks in the FTIR spectra of PE, PS, and PVC were initially identified. For instance, the wavenumber 1471 cm⁻¹ corresponds to the C-H bending vibrations in PE, wavenumbers ranging from 2084 cm⁻¹ to 2026 cm⁻¹ indicate C-H stretching vibrations associated with the aromatic ring and alkyl groups in PS, and a strong peak around 610 cm⁻¹ is indicative of stretching vibrations of C-Cl bonds in PVC. The indexed peaks and the underlying mechanisms for their formation are elaborated upon in Table S2. Furthermore, the FTIR spectrum of the unaltered samples for each polymer is illustrated in Fig. 4, providing a baseline for comparative analysis.

This study examined the impact of the digestion method on FTIR spectral peaks to optimize microplastic analysis. Transmittance changes relative to intact polymers were analyzed across protocols. The PCI quantified treatment effects, where positive values reflect increased transmittance (reduced absorbance), while negative values indicate decreased transmittance with elevated absorbance.

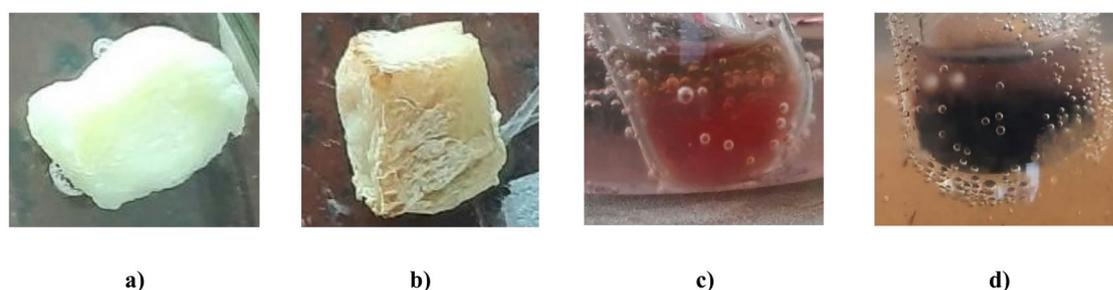


Fig. 3. Fresh beef samples after exposure to (a) H_2O_2 (12 h, 60 °C), (b) Fenton's reagent (6 h, 60 °C), (c) NaOH (6 h, 40 °C), and (d) HCl (6 h, 40 °C).

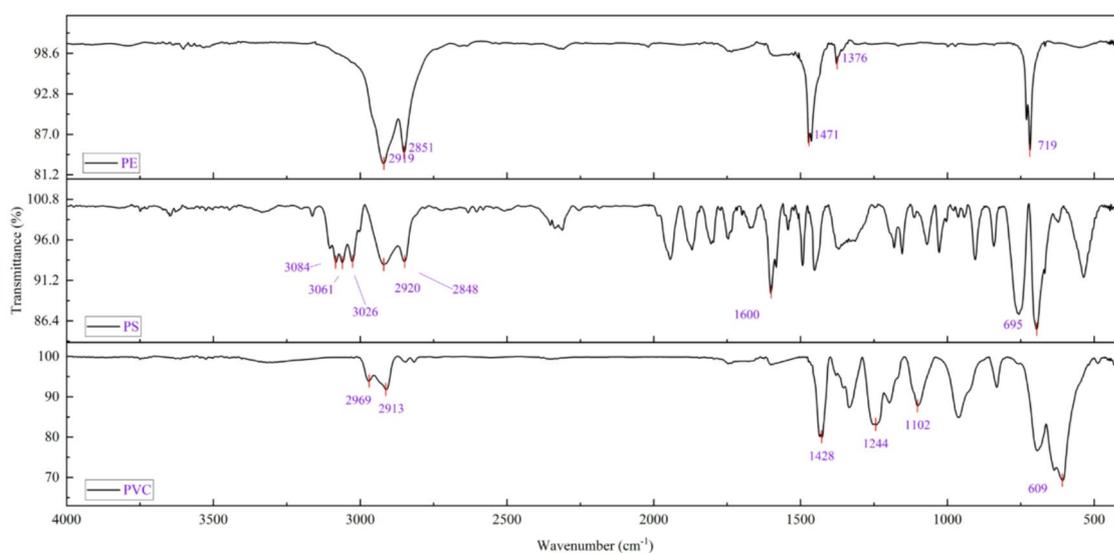


Fig. 4. FTIR spectra of intact PE, PS, and PVC with indexed absorption bands.

An increase in transmittance generally indicates the decomposition or breakdown of the polymer²⁴. Conversely, a decrease in transmittance typically signifies the formation of new functional groups, chemical reactions resulting in new structures that lead to increased IR light absorption, or a higher concentration of specific compounds^{25,26}. Ideally, the optimal digestion process is characterized by minimal changes in transmittance.

The structural changes induced in PE by various chemical treatments were assessed using FTIR spectroscopy (Fig. 5a), with the resulting spectra and a corresponding PCI plot for key wavenumbers presented in Fig. 6. The PCI provides a quantitative assessment of the relative changes in peak intensities compared to the control PE sample (Figs. 5a and 6a). Exposure to NaOH at 40 °C for 6 h resulted in a minimal shift in peak intensities, indicative of negligible structural change. H_2O_2 treatment at 60 °C for 12 h induced a slightly more pronounced change, yet the PCI values suggest a limited overall effect. HCl treatment at 60 °C for 6 h resulted in the most significant PCI deviations, particularly decreasing transmittance across CH_2 symmetric and asymmetric C-H stretching, C-H bending, CH_3 symmetric bending, and split CH_2 rocking vibrations. The 719 cm^{-1} band, associated with split CH_2 rocking vibrations, was most sensitive to these treatments, making it a useful diagnostic marker for PE degradation.

The susceptibility of PS to chemical degradation was investigated through FTIR spectroscopy (Fig. 5b). Exposure to H_2O_2 at 60 °C for 12 h resulted in the most significant spectral alterations (Fig. 6b), with a pronounced decrease in peak intensities throughout the fingerprint region, indicating substantial structural disruption. HCl at 40 °C for 6 h induced minimal spectral changes. Treatment with HCl at 60 °C for 6 h increased PCI values, signifying a reduction in absorbance. The effects of Fenton's reagent (6 h, 60 °C) were less pronounced than those of H_2O_2 .

FTIR analysis of PVC samples exposed to various chemical treatments revealed substantial changes compared to untreated PVC (Fig. 5c). The PCI predominantly exhibited positive values (Fig. 6c), indicating increased transmittance and suggesting polymer decomposition. Treatment of PVC with H_2O_2 for 12 h at 60 °C caused a significant reduction in transmittance across a broad spectral range, particularly in the fingerprint region. NaOH treatment (6 h, 60 °C) increased transmittance, especially below 1500 cm^{-1} , indicating dehydrochlorination and

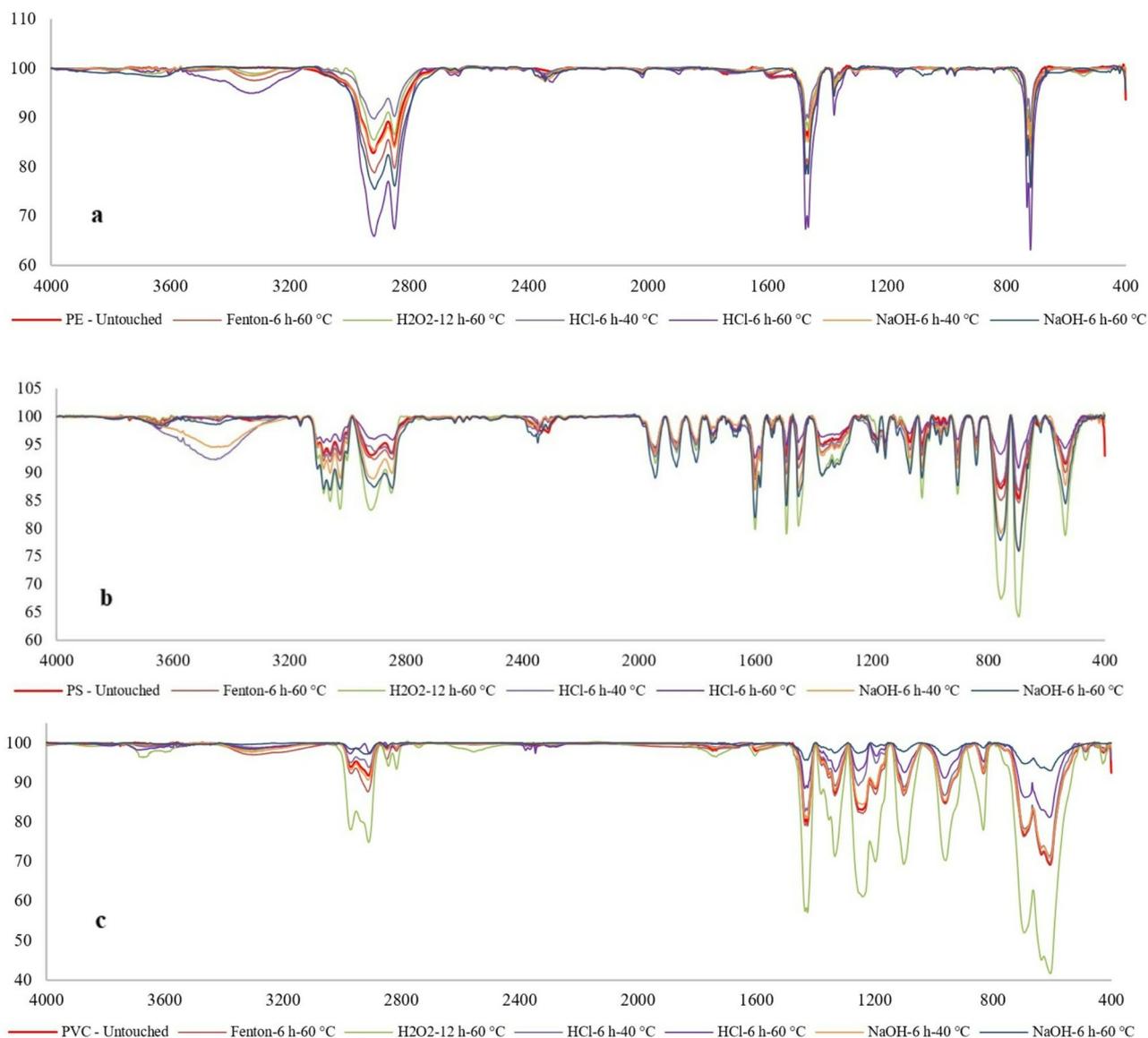


Fig. 5. Effect of digestion on (a) PE-MPs, (b) PS-MPs, and (c) PVC-MPs.

loss of IR-absorbing components. HCl treatment (6 h, 60 °C) increased transmittance, while Fenton's reagent (6 h, 60 °C) and NaOH (6 h, 40 °C) treatments showed minimal changes.

The remaining treatments induced less pronounced but notable changes in the FTIR spectra. HCl treatment (6 h, 60 °C) increased transmittance; in contrast, Fenton's reagent (6 h, 60 °C) and NaOH (6 h, 40 °C) treatments showed minimal changes, indicating a limited impact on PVC's structure.

Analysis of transmittance percentage variations using the PCI revealed the effectiveness of each treatment protocol. As illustrated in Fig. 7, NaOH at 40 °C for 6 h was the most effective treatment, yielding a net negative PCI effect of 7.52% across all polymers, followed by Fenton's reagent (6 h, 60 °C) and HCl (6 h, 40 °C). PVC exhibited lower resistance than PE and PS across various scenarios (with a 56.3% PCI value), particularly for longer durations, with PS also showing reduced resistance to a lesser extent. Higher temperatures (60 °C) when using acid or base caused more significant polymer damage.

Discussion

Efficacy of chemical digestion in reducing organic matter content

According to Fig. 2, the minimal difference in removal percentages between 6 and 12 h suggests that 6 h is optimal for meat digestion with both HCl and NaOH. The Fenton process, characterized by high reactivity over a short time frame, necessitates precise and rapid pH control to prevent $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$ precipitation, making it unsuitable for durations exceeding 6 h.

The visual observations align with previous findings that alkaline solutions are highly effective for tissue digestion²⁷. NaOH's gradual dissolution and production of a transparent pink liquid (Fig. 3c) support its suitability for microscopic inspection, with performance ranked as $\text{NaOH} > \text{HCl} > \text{H}_2\text{O}_2 > \text{Fenton's reagent}$.

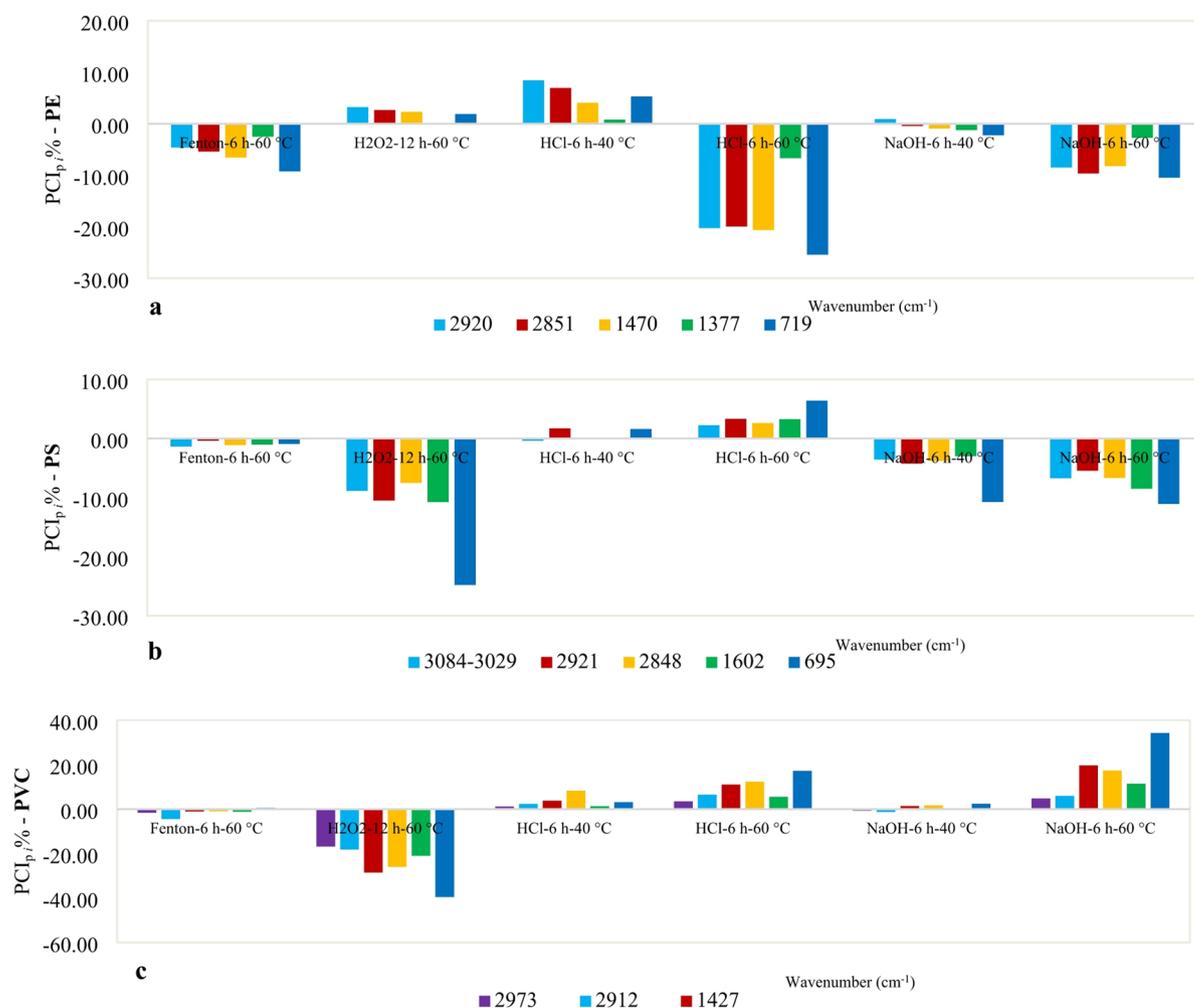


Fig. 6. Impact of digestion protocols on PCI values for (a) PE, (b) PS, and (c) PVC microplastics.

H₂O₂'s bleaching effect²⁸ and the Fenton process's yellowing due to iron deposition^{12,28} further differentiate their impacts on sample appearance. Overall, NaOH-treated samples were visually superior for microscopic analysis, confirming the effectiveness of alkaline digestion methods²⁷.

Effect of chemical digestion on MPs

The comparative analysis of PE structural changes following various chemical treatments, as assessed by FTIR spectroscopy and quantified using the PCI, reveals distinct degradation behaviors dependent on the chemical environment. Exposure to NaOH at 40 °C for 6 h resulted in negligible alterations in the FTIR spectra and minimal PCI deviations, indicating limited structural impact on the PE matrix. In contrast, HCl treatment at 60 °C for 6 h induced substantial spectral changes, with significant decreases in transmittance at key wavenumbers corresponding to CH₂ symmetric and asymmetric C–H stretching, C–H bending, CH₃ symmetric bending, and particularly the split CH₂ rocking vibrations at 719 cm⁻¹. The pronounced sensitivity of the 719 cm⁻¹ band underscores its effectiveness as a diagnostic marker for PE degradation, reflecting surface roughening and disruption of crystalline order^{29,30}. Furthermore, while H₂O₂ at 60 °C for 12 h caused moderate spectral changes, the overall PCI values suggest its degradative effect is less pronounced than that of HCl under the conditions studied. Collectively, these results demonstrate that acid treatment, specifically with HCl, leads to the most significant degradation of PE, surpassing the effects of oxidative and alkaline environments^{9,30,31}.

The FTIR spectroscopy and PCI analysis results demonstrate that PS exhibits markedly different degradation behaviors depending on the chemical environment and treatment conditions. The substantial spectral alterations observed following exposure to H₂O₂ at 60 °C for 12 h confirm that PS is particularly susceptible to oxidative damage, leading to considerable structural disruption. This contrasts with the minimal impact of HCl at 40 °C for 6 h, which reaffirms PS's inherent resistance to moderately acidic environments. However, the increased PCI values resulting from HCl treatment at 60 °C for 6 h suggest that under more forcing conditions, the interplay between acidity and temperature has corrosive effects³⁰ and the decomposition of the polymer matrix²⁴. The effects of Fenton's reagent (6 h, 60 °C) were less pronounced than those of H₂O₂. These findings highlight the

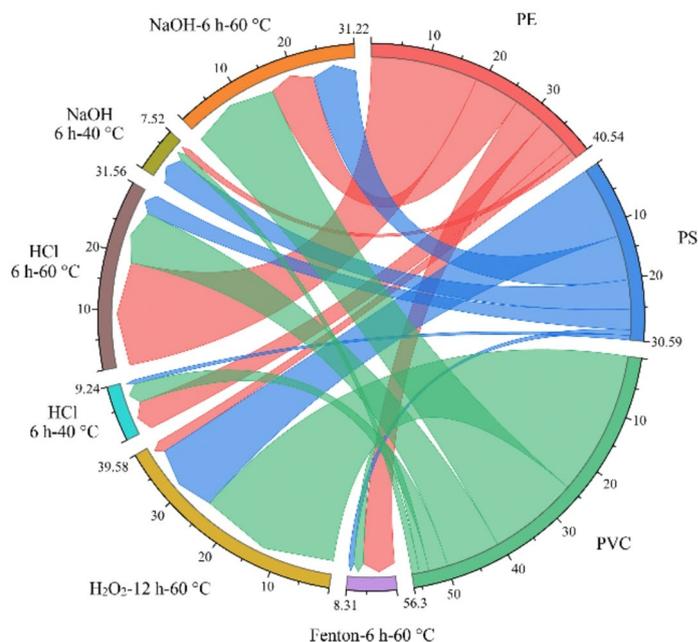


Fig. 7. Cumulative impact of digestion protocols on FTIR spectra of PE, PS, and PVC, as assessed by PCI (showing the total PCI score for each polymer and the percentage contribution of each scenario).

susceptibility of PS to prolonged exposure to oxidizing agents like H_2O_2 , while highlighting a complex interplay between temperature and acidity in mediating its long-term stability.

FTIR analysis of PVC samples exposed to various chemical treatments revealed substantial changes compared to untreated PVC. The PCI predominantly exhibited positive values, indicating increased transmittance. This suggests polymer decomposition or breakdown²⁴. Treatment of PVC with H_2O_2 for 12 h at 60 °C caused a significant reduction in transmittance across a broad spectral range, particularly in the fingerprint region, indicating extensive material degradation. This aligns with Karami et al.³⁰, who observed decreased intensity in the C-Cl stretching band, suggesting chemical modifications and the formation of new IR-active species^{25,26}. Conversely, the NaOH treatment (6 h, 60 °C) increased transmittance, especially below 1500 cm^{-1} , indicating dehydrochlorination and loss of IR-absorbing components.

The remaining treatments induced less pronounced but notable changes in the FTIR spectra. HCl treatment (6 h, 60 °C) increased transmittance, potentially due to reduced functional groups or structural denaturation³⁰. In contrast, Fenton's reagent (6 h, 60 °C) and NaOH (6 h, 40 °C) treatments showed minimal changes, indicating a limited impact on PVC's structure. These findings highlight PVC's sensitivity to specific chemical environments and varying degradation pathways.

The analysis of transmittance percentage variations using the PCI reveals the effectiveness of each treatment protocol. As illustrated in Fig. 7, NaOH at 40 °C for 6 h is the most effective treatment, yielding a net negative PCI effect of 7.52% across all polymers, followed by Fenton's reagent (6 h, 60 °C) and HCl (6 h, 40 °C). Notably, PVC exhibits lower resistance than PE and PS across various scenarios (with a 56.3% PCI value), particularly for longer durations, with PS also showing reduced resistance to a lesser extent. Higher temperatures (60 °C) when using acid or base can cause more significant polymer damage, underscoring the importance of temperature in these treatments. In chemical digestion methods for removing organic matter, meat, NaOH, and HCl are preferred for microplastic analysis. Specifically, NaOH (6 h, 40 °C) is recommended as the primary option, with HCl as a secondary choice. Conversely, Fenton's reagent and H_2O_2 are not recommended for analyzing microplastics in meat due to their limited effectiveness in this context.

Conclusion

PCI offers a practical way to understand how different digestion methods affect microplastics, helping researchers compare their impact on various polymers. This study shows that careful selection of digestion protocols is crucial for accurate food microplastic analysis. By refining these methods, such as using gentler treatments like NaOH (6 h, 40 °C), which showed minimal interference (7.52% PCI effect), we can better measure microplastics and trace how they enter our diets. These insights are vital for assessing risks and designing strategies to reduce contamination.

The urgency of microplastic pollution calls for a deeper investigation into its effects on health and the environment. Future work should expand this approach to other foods and identify contamination sources, supporting global efforts to curb pollution. PCI's ability to compare how common methods alter different polymers makes it valuable for harmonizing research and improving standards.

Data availability

The datasets analyzed during this study are available from the corresponding author upon reasonable request.

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Author contributions

Saber Entezari: conceptualization; investigation; resources; writing—original draft; writing—review and editing. Hossein Ganjidoust: conceptualization, methodology, investigation, supervision (supervisor).Yadollah Yamini: investigation, supervision (advisor).Bita Ayati: investigation, supervision (advisor).

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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