



Biodegradation of Polystyrene Paper Using Chewing Insects

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Abstract

Background: Plastic pollution, particularly from polystyrene, has emerged as a serious environmental concern, prompting growing interest in its biodegradation. We investigated the potential of four chewing insects; mealworm (*Tenebrio molitor*), superworm (*Zophobas morio*), American cockroach (*Periplaneta americana*), and cricket (*Gryllus bimaculatus*) to biodegrade polystyrene paper (PSP).

Methods: Using a randomized complete block design, four chewing insects were divided into two groups after 48-hour fasting with water. One group received PSP only; the other received PSP mixed with tapioca starch. Weight loss of PSP was recorded after 72 hours.

Results: *Z. morio* demonstrated the highest degradation efficiency for PSP (92.10%), followed by *P. americana* (32.17%). When tapioca starch was added, *Z. morio* remained the highest effective (95.45%), followed by *T. molitor* (59.15%). Supplementing starch significantly enhanced degradation rates ($P < 0.05$). Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) revealed signs of depolymerization, oxidation, and surface cracking. FTIR indicated new functional groups of carbonyl ($C=O$, 1650 cm^{-1}) and hydroxyl ($O-H$, $3200-3550$ and $3584-3700\text{ cm}^{-1}$).

Conclusion: Certain chewing insects, especially *Z. morio* and *T. molitor*, possess strong potential for PSP degradation, likely aided by their gut microbiota. Notably, this study is the first to report PSP degradation by *P. americana* and *G. bimaculatus*. Further research is needed to explore the microbial mechanisms within insect guts that facilitate plastic biodegradation.

Keywords: Biodegradation; Polystyrene; Plastic; Chewing insects

Introduction

Plastic, a ubiquitous synthetic material, plays a pivotal role in daily life, contributing to various industries and applications. The global production of plastics surged to 390.7 million tons in 2021, with packaging and construction being the largest markets. Projections indicated that plastic production may reach a staggering 1,600 million tons annually by 2050 (1, 2). Packaging alone accounted for 146

million tons, constituting 42% of total plastic consumption (3). Notably, the surge in food delivery and services has significantly contributed to the solid waste stream, predominantly composed of polystyrene (PS) and polypropylene (PP) plastic containers, collectively making up around 75% of total packaging waste (4).



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Among these plastic pollutants, PS stands out as a major environmental concern, with an annual production exceeding 20 million tons (5). Its widespread use has led to PS becoming a major contributor to soil, river, lake, and ocean pollution (6). The environmental impact was further exacerbated by the formation of microplastics (<5 mm) in the oceans, where PS constitutes a significant portion (7, 8). Study have revealed that plastic particles, including fragments, film, and foam, constitute a substantial portion of the digestive tract contents of various fish species, highlighting the pervasive nature of plastic pollution in aquatic ecosystems (9).

The conventional disposal methods for these plastic wastes, including incineration, landfilling, and ocean dumping, posed severe environmental threats (10, 11). Due to the prolonged decomposition time of plastics, their accumulation in ecosystems has far-reaching detrimental effects on the environment and human society (12). In response to this crisis, study has explored the degradation of plastics, such as polyethylene (PE) and PS, utilizing microorganisms like bacteria and fungi (13). Biodegradation, a process involving the use of living organisms, offers a more environmentally friendly alternative compared to chemical and physical disposal methods (14). It represents a sustainable approach for plastic waste disposal, where microbial enzymes initiate the degradation process, breaking polymer bonds and transforming large, hydrophobic polymers into smaller, hydrophilic monomers that can biodegrade within microbial cells (15).

Despite the scientific consensus suggesting that PS degradation requires pre-treatment such as photolytic or thermolytic cleavage of -C-C- bonds before biodegradation (3, 16), recent studies challenge this notion. Notably, the guts of yellow mealworm larvae (*T. molitor*) and the gut microbial flora of *Plesiophthalmus davidis* larvae exhibited rapid PS degradation (17-19). Superworm larvae (*Zophobas morio*) and their gut bacterium *Pseudomonas* sp. strain DSM 50071, as well as *Rhizopus arrhizus* lipase and α -amylase, have also demonstrated PS-degrading capabilities (20-24). This di-

versity in PS-degrading organisms suggests a potential avenue for enhancing the efficiency of PS biodegradation by utilizing various species.

We aimed to investigate the biodegradation of polystyrene by chewing insects including cricket (*G. bimaculatus*), mealworm (*T. molitor*), superworm (*Z. morio*), and American cockroach (*P. americana*) which possess chewing mouthparts and digestive enzymes potentially capable of breaking down polystyrene, offering a sustainable approach to reducing plastic waste.

Materials and Methods

Preparation of Polystyrene Material

Polystyrene paper (PSP) is a foam used for food packaging (SITEX Foam, Seatex Industry Corporation, Thailand). The unused PSP was separated into two groups; the first group was cut into 1×1 cm and weighed for 0.1 g. The other was cut into 1×1 cm weighed 0.09 g and then mixed with 0.01 g of tapioca starch (New Grade, Thai Wah, Nakhon Pathom Province, Thailand)

Preparation and Collection of Chewing Insects

Chewing insects used in the experiments were obtained from reared and bred adult (F_1) insects until the F_2 generation were obtained for further experiments. In April 2022, *G. bimaculatus* adults sourced from a farm in Maha Sarakham Province, Thailand were reared for egg production. Eggs were incubated at 25–28 °C, and the resulting nymphs were raised on a substrate until reaching the target developmental stages: 4 weeks as nymphs and 4 weeks post-adult emergence. Larvae of *T. molitor* and *Z. morio* were obtained from a pet food supplier in Maha Sarakham Province, Thailand. Moreover, *P. americana* were caught from the local fresh-food market in Maha Sarakham Province by modified jar traps (25). The traps were placed at 6:00 p.m. and collected at 6:00 a.m. the next day. Cockroach adults were bred for ootheca production. Oothecas were incubated at 25–28 °C, and the emerging nymphs were reared on a substrate until they reached 8 weeks as nymphs and an additional

8 weeks following adult emergence. All insects were reared in the laboratory of the Faculty of Public Health, Mahasarakham University. The temperature and relative humidity were set at 25-28 °C and $57 \pm 3\%$ RH, respectively. The insect food was ready to use powder feed for insects (J80, Lee Pattana Feed, Thailand) and contained 21% protein, and 4% fat, which has been tested for non-microplastic contamination (9).

Polystyrene Degradation by Chewing Insects

This study employed a randomized complete block design (RCBD) with two experimental groups. All insects (F2 generation) were fasted for 48 hours prior to the experiment but provided with drinking water. In the first group, insects were exposed to 0.1 g of polystyrene paper (PSP). In the second group, they were exposed to a mixture of 0.09 g PSP and 0.01 g tapioca starch. Each block contained 25 insects of similar size and weight ($\pm 5\%$), and the experiment was conducted over 72 hours. Insect frass was collected every 24 hours, with 10 replications per treatment. The control group did not put insects into the blocks. PSP was collected after biodegradation of chewing insects, washed in ethanol and dried at 40 °C for 24 h, and lost weight was determined by an electronic balance (Sartorius, PRACTUM224-1S, Germany) (26).

Digestion Method

All described steps were performed under a fume hood with synthetic-free clothing, and gloves were worn at all times. The samples were put in 250 mL glass beakers which were covered by watch glasses to prevent contamination. Sodium hydroxide solution (NaOH 1 mol L⁻¹, KemAus™, Australia) was added according to the weight of the sample in each beaker (27).

The solutions were heated at 50 °C on a hot plate for 15 min and mixed with a stirring bar. Next, appropriate volumes of nitric acid (HNO₃ 65%, KemAus™, Australia) and ultrapure water were added, then the samples were heated for another 15 min at 50 °C (final concentration of HNO₃: 10 mol L⁻¹). The temperature

was then increased to 80 °C during 15 min to ensure the removal of more resistant suspended solids. For filtration, the samples were diluted at a 1:2 ratio with ultrapure water heated to 80 °C and filtered on a filter paper No.42 (Ø 47 mm, 2.5 µm pore size, Whatman, England). The beakers and filtration devices were also rinsed with warm ultrapure water to ensure no loss of material.

The sample derived from the first two digestion steps remained on the filters with no trace of mineral residues, the filter paper was placed in 150 mL glass beakers and poured with 20 mL of 1 mol L⁻¹ NaOH, and the solutions were heated at 50 °C until the filters were dissolved completely remove the filter paper and rinsed with warm ultrapure water to ensure no loss of material. The samples then were diluted with 100 mL of 80 °C ultrapure water and filtered on glass microfiber filter (GF/C) (Ø 47 mm, 1.2 µm pore size, Whatman, England) which were dried at room temperature and subsequently analyzed (27).

Fourier Transform Infrared Spectroscopy (FTIR) Analysis of the insect frass

The insect frass which went through the process of digestion method, were dried on filter paper at 40°C for 24 hours, and was then analyzed PSP (control) and PSP (processed sample biodegradation) using FTIR (BRUKER, INVENIO-S Internal Transmission RT-DLaTGS, Germany). The absorbance ranged from 4,000 cm⁻¹ to 650 cm⁻¹ with a scan resolution of 4 cm⁻¹ for the FTIR. A background scan was performed each time before the sample was scanned, and the final sample spectrum was subtracted from the background scan value.

Scanning Electron Microscopy (SEM)

The SEM was used to study the physical characteristics of PSP in the changes of surface characteristics before and after the biodegradation process. PSP (control) and PSP (processed sample biodegradation) were coated with gold, and the surface topography of biodegradation-treated or untreated PSP was observed under the SEM (HITACHI, TM 4000 PLUS), and was then imaged.

Statistical analysis

Institution Animal Care and Use Committee (IACUC) of Mahasarakham University, Maha Sarakham, Thailand, approved the use of animals under this study (IACUC-MSU-28/2021). All data were subjected to one-way analysis of variance (ANOVA) followed by Fischer's LSD post hoc test using Statistical Package for Social Science (SPSS) version 21 (IBM Corp., Armonk, NY, USA). Statistical significance was considered at $P < 0.05$.

Results

Comparative Biodegradation Efficiency of PSP by Chewing Insects

This study compared the biodegradation efficiency of PSP among various chewing insects, including nymphs and adults of *G. bimaculatus* and *P. americana*, as well as adults of *T. molitor* and *Z. morio*. Weight loss of PSP and PSP mixed with tapioca starch was quantitatively measured after 72 hours. Table 1 presents the PSP weight loss observed for each insect species.

Table 1: Biodegradation of PSP and PSP–tapioca starch mixture by chewing insects (percentage weight loss)

Insect Type	Percentage weight loss of plastic (%)	
	PSP	PSP–tapioca starch mixture
<i>T. molitor</i> Adult	31.58	59.15
<i>Z. morio</i> Adult	92.10	95.45
<i>P. americana</i> Adult	32.17	48.38
<i>P. americana</i> Nymph	23.24	34.22
<i>G. bimaculatus</i> Adult	28.56	37.23
<i>G. bimaculatus</i> Nymph	26.44	30.20

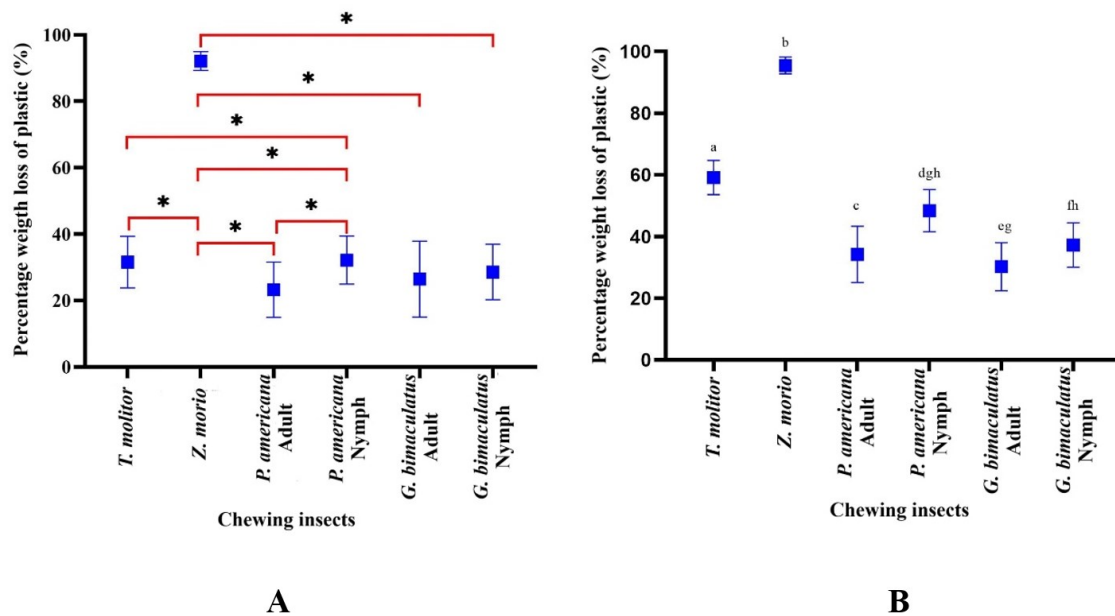


Fig. 1: Mean percentage weight loss of PSP (A) and PSP–tapioca starch mixture (B) degraded by chewing insects. * Significant difference ($P < 0.05$), and the means with different letters in each row were significantly different ($P < 0.05$)

Among these chewing insects, *Z. morio* had the highest biodegradation of PSP followed by *P. americana* adult. When tapioca starch was added, *Z. morio* remained the highest effective, followed by *T. molitor*. Significant differences ($P < 0.05$) were observed in the degradation efficiency between PSP and PSP mixed with tapioca starch, as well as among the different chewing insect species. Moreover, Fig. 1 compares the mean percentage weight loss of PSP and PSP mixed with tapioca starch among *T. molitor*, *Z. morio*, *P. americana*, and *G. bimaculatus*. Additionally, *Z. morio* exhibited the highest degradation efficiency for both PSP types, with significant differences compared to the other insects ($P < 0.05$).

FT-IR Analyses of PSP Biodegradation by chewing insects

FT-IR analysis was performed to detect chemical structural changes in PSP before and after biodegradation. Characteristic absorption peaks of carbonyl (C=O) at $1,650\text{ cm}^{-1}$ and hydroxyl (O-H) at $3,200\text{--}3,550\text{ cm}^{-1}$ and $3,584\text{--}3,700\text{ cm}^{-1}$ were observed in the treated samples but were absent in the control group, indicating that PSP biodegradation mediated by chewing insects had occurred (Fig. 2).

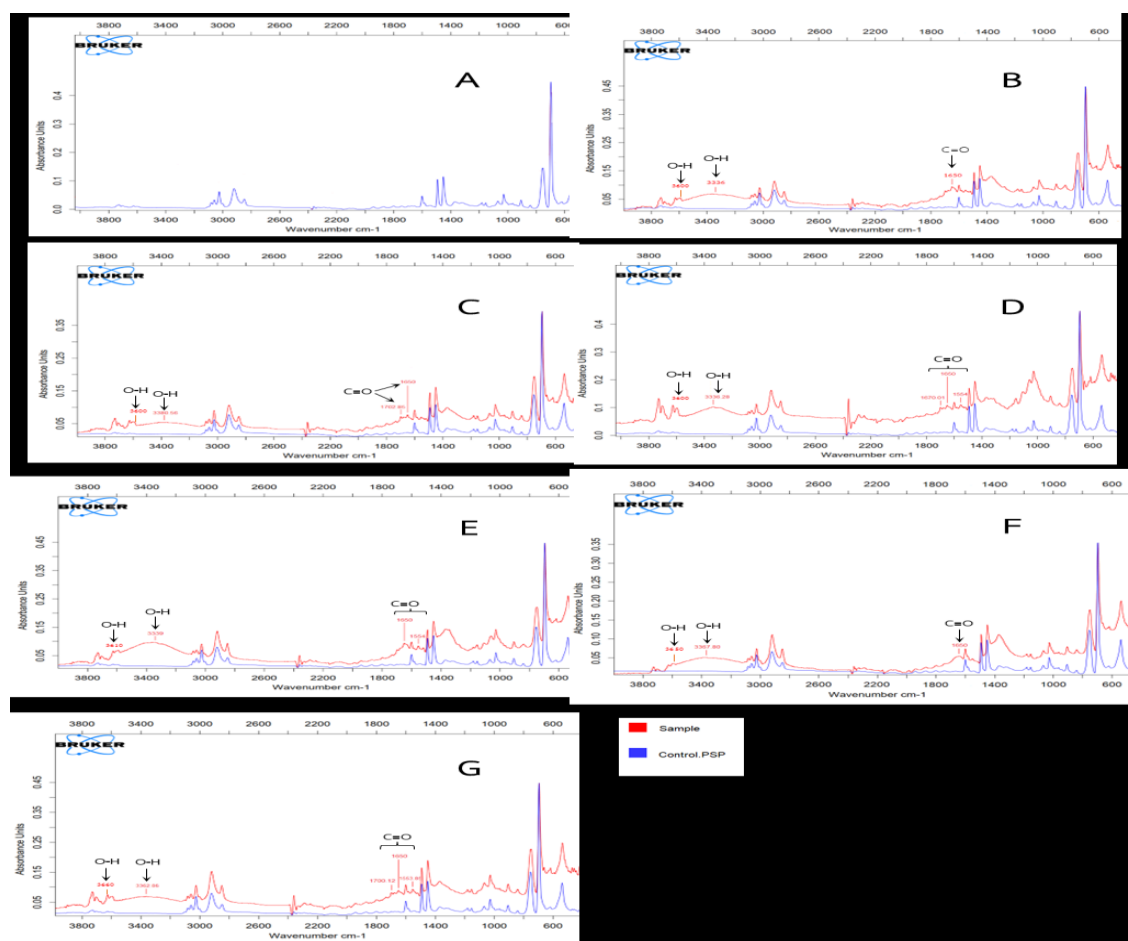


Fig. 2: FT-IR analysis of chemical structural changes during PSP biodegradation.

A = control group; B–G = FT-IR spectra of PSP biodegraded by *T. molitor*, *Z. morio*, *P. americana* adult, *P. americana* nymph, *G. bimaculatus* adult, and *G. bimaculatus* nymph, respectively. Red lines represent biodegraded PSP, while blue lines represent the untreated control

SEM Analyses of PSP Biodegradation by Chewing Insects

Before the experiment, PSP plastic exhibited a smooth surface with a well-ordered arrangement of polymer pellets. However, after biodegradation, notable surface alterations were observed. The

once-smooth surface became rough and was marked by numerous cavities surrounding the sample. SEM analysis confirmed these changes, revealing a transition from the original smooth morphology to a rough, pitted surface following exposure to chewing insects (Fig. 3).

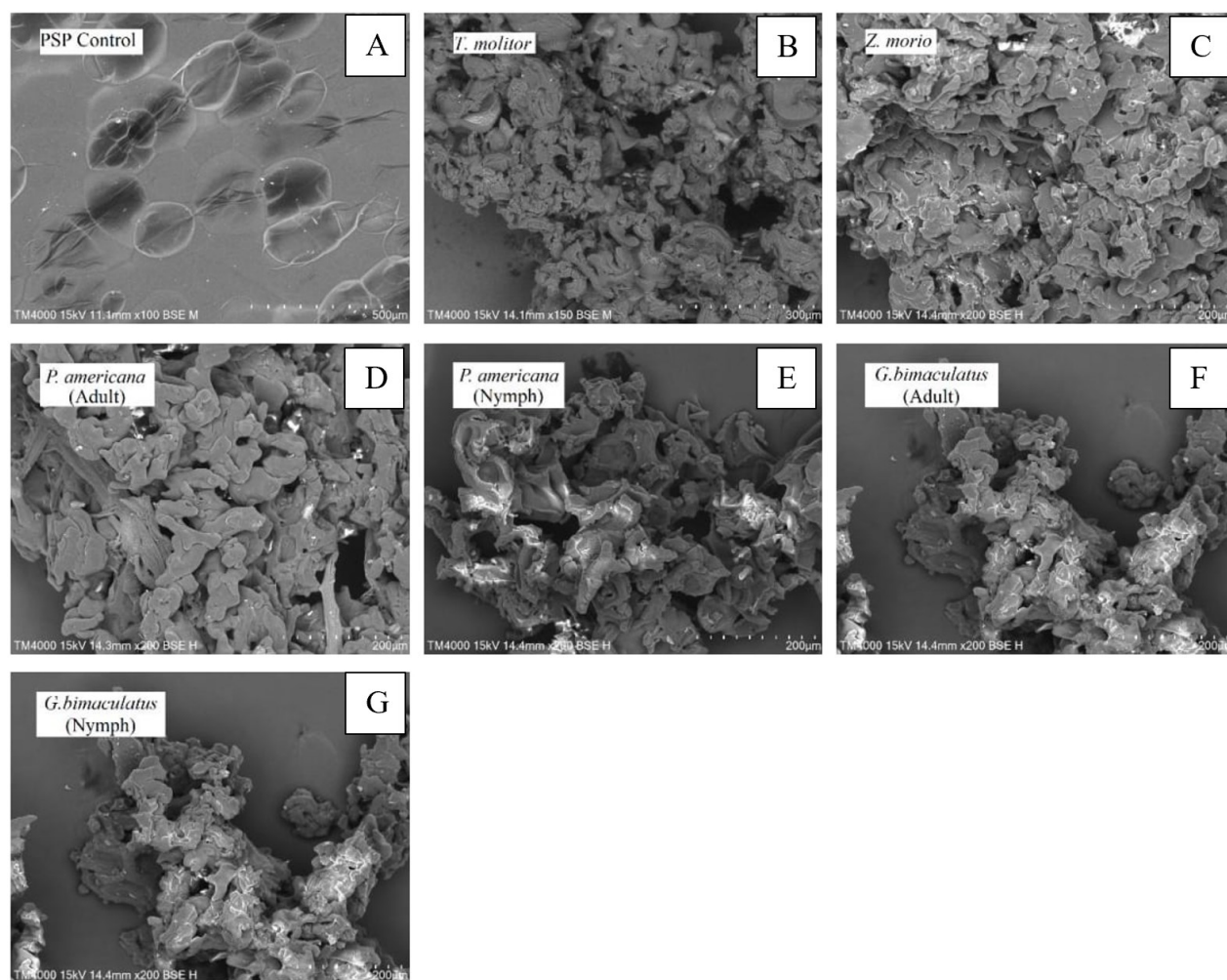


Fig. 3: Scanning Electron Microscopy (SEM) images of polystyrene paper (PSP) surfaces (A) Untreated PSP (control), showing a smooth and intact surface. (B–G) PSP surfaces after 72-hour exposure to chewing insects: (B) *T. molitor*, (C) *Z. morio*, (D) *P. americana* adult, (E) *P. americana* nymph, (F) *G. bimaculatus* adult, (G) *G. bimaculatus* nymph. Biodegraded PSP samples display roughened surfaces with cavities, fractures, and structural disruption, indicating active degradation compared to the control.

Discussion

This study highlights the potential of chewing insects such as *T. molitor*, *Z. morio*, *P. americana*, and

G. bimaculatus to biodegrade PSP, a persistent plastic pollutant. *Z. morio* showed the highest degradation efficiency, followed by *T. molitor*, *P. americana*, and *G. bimaculatus*. For the first time, both nymphs and adults of *P. americana* and *G. bimaculatus* were

found to degrade PSP, broadening knowledge of insect-mediated plastic biodegradation. The PS consumption rates of *T. molitor* (12.72 mg/day) and *Z. morio* (7.88 mg/day) were far higher than previously reported (0.12 and 0.58 mg/day, respectively) (17–18, 23). This is also the first report of PS degradation by *P. americana* (adults: 6.45; nymphs: 7.88 mg/day) and *G. bimaculatus* (adults: 4.96; nymphs: 4.02 mg/day), whereas earlier studies documented only polyurethane ingestion by *G. bimaculatus* adults (0.28 mg/g/day) with no PS degradation (29).

The addition of tapioca starch significantly enhanced PSP degradation across all insect species ($P < 0.05$). This improvement can be attributed to increased palatability and feeding activity, as the non-plastic additive likely stimulated insect appetite and prolonged ingestion. This finding is consistent with previous reports that suggest co-feeding organic substrates can enhance plastic consumption in insects by improving survival and digestion (28).

FT-IR analysis revealed the formation of new functional groups—carbonyl (C=O) and hydroxyl (O–H)—in the PSP samples exposed to chewing insects, which were absent in the control group. The appearance of these peaks indicates oxidative degradation and structural modification of the polymer chains. This result aligns with previous studies involving microbial and fungal degradation of synthetic polymers, where oxidation and depolymerization were identified as key steps in plastic breakdown (30, 31). Furthermore, the transformation of polystyrene from a hydrophobic to a more hydrophilic surface through oxidation has been shown to facilitate microbial colonization and subsequent biodegradation (32).

Furthermore, SEM analysis provided visual confirmation of the surface damage caused by chewing insects. While untreated PSP exhibited a smooth and intact morphology, the biodegraded samples showed rough, pitted surfaces with significant fragmentation. These alterations are indicative of mechanical disruption combined with biochemical degradation, likely mediated by the insect's chewing behavior, digestive enzymes, and gut microbiota. Similar findings were reported by

Chen et al (33), who demonstrated that oxidative mechanisms contribute to polymer chain scission resulting in surface erosion and particle fragmentation.

Taken together, the evidence from this study suggests that chewing insects facilitate PSP biodegradation through a synergistic mechanism involving mechanical fragmentation, oxidative depolymerization, and potential microbial action within the gut. The significantly higher degradation rates observed in this study, particularly in *T. molitor* and *Z. morio*, compared to previous reports, highlight the influence of experimental conditions such as substrate composition, insect developmental stage, and feeding duration. Future research should focus on characterizing the gut microbial communities and enzymes responsible for polystyrene degradation, which could pave the way for biotechnological applications in plastic waste management.

Conclusion

This study highlights the potential of chewing insects such as *T. molitor*, *Z. morio*, *P. americana*, and *G. bimaculatus*—as effective agents for PSP biodegradation. These insects reproduce rapidly, require minimal space, and are low-cost to maintain, making them suitable for scalable applications. While the biodegradation capability of *T. molitor* and *Z. morio* larvae has been previously documented, this study is the first to report significant PSP degradation by *P. americana* and *G. bimaculatus*. The observed efficiency suggests that both mechanical chewing and gut-associated microbial activity contribute to the degradation process. These findings offer a promising biological approach to managing plastic waste and underscore the need for further research into the microbial communities and enzymatic mechanisms within insect digestive systems that facilitate polystyrene breakdown.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission,

redundancy, etc.) have been completely observed by the authors.

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Conflict of Interest

The authors declare that there is no conflict of interests.

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