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Towards Entomoremediation: Plastic Consumption Rates and FTIR-ATR Analysis of Plastic, Frass and Exuviae from *Tenebrio Molitor* Fed with EPS and Maize

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ABSTRACT

Purpose: This study aimed to (1) evaluate the behavior of *Tenebrio molitor* larvae when consuming EPS in the presence of maize grains to identify consumption patterns, and (2) assess the possibility of using FTIR-ATR analysis of untreated samples of plastic, frass and exuviae to determine discernable changes after the bingeing phase of EPS consumption versus the control group.

Methodology: *T. molitor* larvae were obtained from Tuxtla Gutierrez Chiapas, México and the maize grains from Tlaxcala, México. The EPS consumption assays were done once a week and were expressed as the remaining percentage of EPS. FTIR-ATR analysis was performed on samples of plastic, frass and exuvia obtained from the EPS-maize treatments.

Findings: Three distinct phases were identified in EPS consumption: an initial lag phase, a significant bingeing phase, and a stabilization phase with declining consumption. Maize addition triggered a new bingeing phase. FTIR-ATR revealed consistent spectral differences between control and EPS-exposed samples in plastic and frass, indicating that *T.molitor* larvae induce chemical modifications in EPS. EPS-derived compounds were detected in frass but not in exuviae, suggesting excretion of degradation byproducts but not accumulation in the cuticle.

Unique Contribution to Theory, Policy and Practice: Direct FTIR-ATR measurements of untreated samples were sufficiently sensitive to distinguish differences among them, highlighting its utility as a rapid, non-destructive tool for preliminary biodegradation assessment. These results aim to improve entomoremediation strategies and enable more efficient evaluation of larval-mediated plastic degradation.

Keywords: *Entomoremedition, Expanded Polystyrene, FTIR-ATR, Tenebrio Molitor*

JEL Codes: *Q01, Q20, Q24*.

1. INTRODUCTION

Worldwide production of plastics in 2021 reached 390.7 Mt (million tons) (Statista Research Department, 2023) and plastic consumption has quadrupled over the past 30 years, which accounts for 3.9 % of global gas emissions. Plastic waste generation doubled from 2000 to 2019, reaching 353 Mt, of this, only 9% was recycled, 19% incinerated, 50% landfilled, and 22% mismanaged or untreated, of which 6.1 Mt leaked into aquatic environments by 2019 (OECD, 2022). Expanded polystyrene (EPS) is one of the major plastic pollutants worldwide, with global production reaching 21,116 tonnes in 2019, accounting for 4.59% of total plastic production that year (OECD, 2022).

EPS is made by blowing gases into heated polystyrene to produce foamed polystyrene, which is widely used mainly for packaging and for fast food delivery (Salisu and Maigari, 2021). Researchers have demonstrated that chemicals can migrate from EPS packaging into food, posing a potential hazard due to the presence of styrene monomers, plasticizers, and other additives commonly found in this plastic (Meng et al., 2023; Alamri et al., 2021). The International Agency for Research on Cancer (IARC) has classified styrene as a possible human carcinogen, after experimental studies in animal models have demonstrated the carcinogenic potential of styrene oxide, a highly reactive metabolite derived of styrene (Salisu and Maigari, 2021). It has also been shown that microparticles of PS (polystyrene) might cause damage to skin at extremely high concentrations, and could also cause cytotoxicity, hemolysis, and inflammation as particle size decreases (Hwang et al., 2020).

Mealworm is the common name for the larvae of *T. molitor* Linnaeus, a species of darkling beetle, commonly used as poultry-feed and, at an increasing rate, as a source of protein and nutrients for human consumption (Hong et al., 2020; Iyapo et al., 2024). These larvae have been previously proven to consume EPS, and their plastic consumption capacity is influenced by the co-diet to which the larvae is subjected to. Also, different FTIR analysis of the EPS in contact with the larvae and of the frass from it under different conditions have demonstrated chemical changes respectively in their structure, but this has not been shown in the exuvia (molted exoskeletons) of the larvae (Yang et al., 2021, Yang et al 2018; Jiang et al., 2021; Liu et al., 2022; Ilijin et al., 2024).

The increasing rate of plastics disposal, and the increasing appearance of micro and nanoplastics, has led to the need to find alternative ways to deal with them, including entomoremediation, which is the use of insects, or their associated microorganisms or enzymes, to clean pollutants from the environment (Gwenzi et al., 2024). In this way, any scientific knowledge generated that could help in the development and application of proper technologies to decrease the plastic presence in the environment is a step further into a sustainable future. The aim of this work was to determine the influence of co-feeding larvae of *T. molitor* with maize in the consumption rate of EPS; and also, we analyzed through FTIR, the EPS bitten by the larvae, its frass, and exuvia to determine presence or absence of changes due to EPS diet.

2. MATERIALS AND METHODS

2.1. Obtention of *T. molitor* larvae

T. molitor larvae of 1.5 to 2 cm long were obtained from the poultry-feed supplier Crickets Tuxtla (Tuxtla Gutierrez, Chiapas, México). Once received, they were transferred into large plastic trays and acclimatized on oatmeal for two weeks prior to experiments to standardize their gut microbiota, mitigate transport stress effects, and eliminate residual dietary interference when initializing the EPS and maize experiments.

2.2. Collection of maize grains

Maize grains (*Zea mayz*) were collected from San José Aztatla, a community in the municipality of Contla de Juan Cuamatzi, in the state of Tlaxcala, México. They were dried at 40 °C for 24 h prior to use to avoid any trace of humidity that may promote the appearance of fungi or bacteria that could affect the growth of the larvae during the experiments.

2.3. Remaining EPS essays

Expanded polystyrene (EPS) was obtained from common package. For the experiments, 260 mL square glass tanks were used, each tank was filled with a two-finger-wide layer of earth-humus substrate, which was sterilized by heating at 75 °C for 24 hours prior to use. Three experimental treatments with 3 repetitions each were established: A) control EPS without larvae; B) 3 g of EPS + 3 g of maize with 120 larvae; and C) 3 g of EPS with 120 larvae. The tanks were equipped with a thermal blanket and a manual temperature controller, maintaining a constant temperature of 28 ± 2 °C. For the duration of the 11-week experiment, all the treatments received light moisture once per week. We measured the percentage of remaining EPS with the equation below:

$$Remaining\ percentage\ of\ EPS\ (\%)=\frac{Weekly\ weight\ of\ EPS\times 100}{Initial\ weight\ of\ EPS}$$

Larvae were not replenished during the experiment in order to isolate the effects of EPS in the larvae, avoiding microbiota variations and the introduction of larvae of different ages or sizes. To reduce cannibalism, pupae and adult beetles were transferred to separate containers. Prior to weighing, EPS samples were cleaned with a brush and measured using an analytical balance (OHAUS, model Adventurer, AX324). Maize was also weighed but not replenished throughout the experimental period, to avoid maize-fed microbial succession or fresh nutrient acquisition which could artificially alter EPS consumption rates. The consumption rate slopes (% of EPS consumption per week, %EPS/w) and coefficients of determination (R^2) were obtained using the tendency line function from WPS Spreadsheets ver 12.2.0.22549 from WPS Office 2025.

2.4. FTIR analysis

FTIR-ATR spectroscopy in the near-infrared region was conducted using a Perkin Elmer instrument (Part number L1280140) on samples including EPS bitten by the larvae, larval frass, and exuviae, including corresponding controls. Analyses were carried out to samples from larvae fed with maize both in the presence and absence of EPS. Using FTIR-ATR allowed the study of the samples as they occurred in the experiment without further treatment, avoiding possible physical or chemical modifications that came from interactions with any reactants for preparation.

3. RESULTS

3.1. EPS consumption rates

In the experiment without maize, three distinct phases of plastic consumption were observed through week 9; following the addition of 3 g of maize at week 10, a fourth phase emerged and persisted through week 11 (Figure 1). The first phase lasted from week 1 to 3, which showed a reduction in EPS weight of 5.1 % with a slight slope (m_1 = -1.9329 %EPS/w, R^2 = 0.8626); the second phase, from week 4 to 5, had a higher plastic weight reduction of 10.5 % and a steeper slope (m_2 = - 5.2564 %EPS/w, R^2 = 0.9637); and the third phase, between weeks 6 and 9, had a plastic weight reduction of 2.9 %, and showed a slight leveling of the slope (m_3 = -0.7439 %EPS/w, R^2 = 0.9824). From week 10 to 11, three grams of maize were added and a fourth phase developed in which a slightly steeper slope of consumption appeared (m_4 = - 2.8585 %EPS/w, R^2 = 0.8766), with an EPS weight reduction of 5.7 %. The complete experiment had a total EPS weight reduction of 24.2 % from day 0 to week 11 (Figure 1), after which the plastic was not completely consumed. The negative sign of the slopes represents a decrease in weight of the plastic as time progresses.

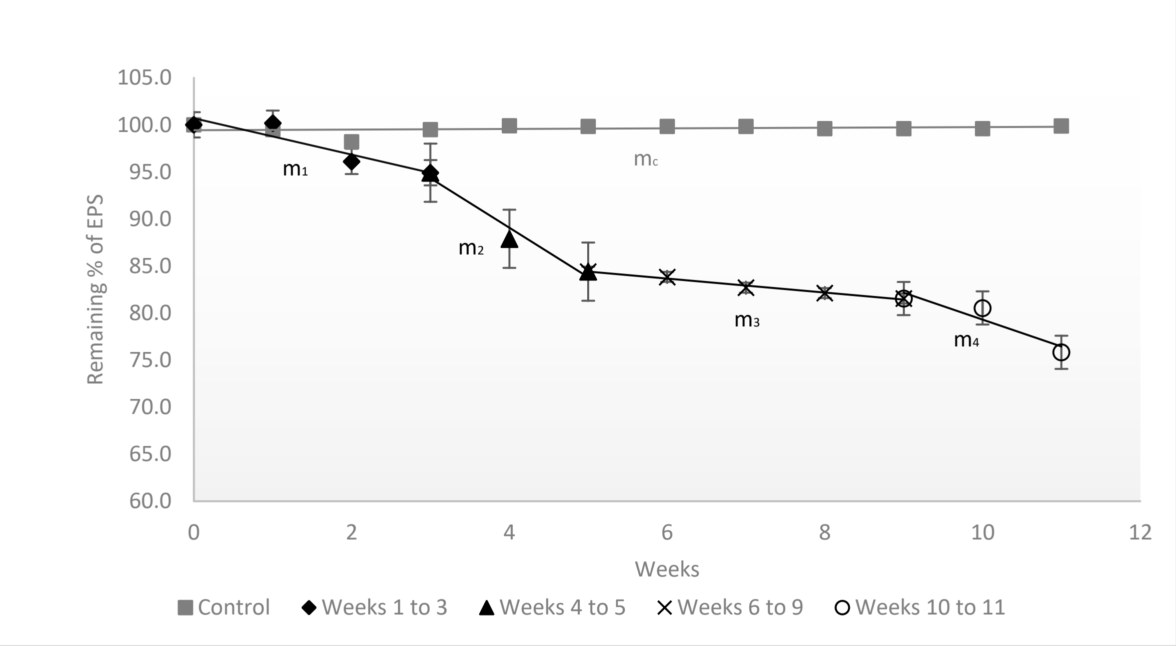

Figure 1. Remaining percentage of EPS in the absence of maize. Feeding behavior of *T. molitor* with EPS in the absence of maize (weeks 0 to 8), and when maize was added (weeks 9 to 11). The EPS weight loss is presented as a percentage of its initial weight.

The second experiment had EPS and maize from day 0, and three phases of consumption of plastic were observed (Figure 2). The first phase, from week 1 to week 2, had a plastic weight loss of 0 % with an almost perfect horizontal slope (m_1 = 0.1904 %EPS/w, R^2 = 0.477). The second phase, from week 3 to 4, had a weight loss of 19.7 % and a pronounced slope (m_2 = -10.032 %EPS/w, R^2 = 0.9791). Lastly, a third long phase from week 5 to 11, which had a weight loss of 14.9 % and a lesser steep slope (m_3 = -2.0265 %EPS/w, R^2 = 0.9774) compared to the second phase. The total EPS weight loss of this experiment was of 34.6 % from week 1 to 11, and similarly to the first case, the plastic was not completely consumed at the end of the experiment. The negative sign of the slopes represents a decrease in the weight of the plastic across time. Control experiments without larvae showed no weight loss throughout the whole experiment (m_c = 0.033 %EPS/w, R^2 = 0.0628) in both the absence and presence of EPS (Figure 1 and 2).

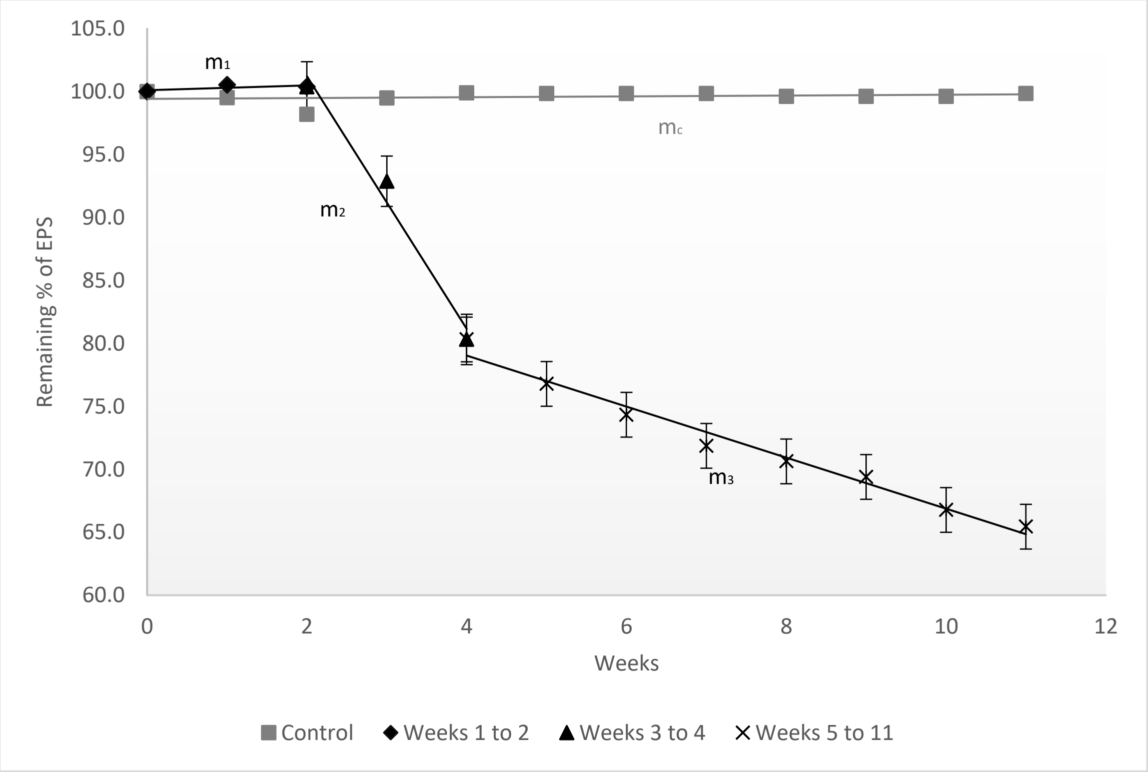

Figure 2. Remaining percentage of EPS in the presence of maize. Feeding behavior of *T. molitor* with EPS in the presence of maize (weeks 0 to 8), and when maize was added (weeks 9 to 11). The EPS weight loss is presented as a percentage of its initial weight.

3.2 FTIR analysis of the EPS

FTIR-ATR spectroscopy was employed to determine potential structural changes in expanded-polystyrene (EPS) following exposure to *T. molitor* larvae across 5 weeks of contact with the plastic. The IR spectrum of the control polystyrene is described as follows: A small band at 3025 cm^{-1} corresponding to C–H aromatic stretching or tension; a medium band at 2920 cm^{-1} ($-CH_2$

asymmetric stretching or tension); a small narrow band at 2807 cm^{-1} (-CH_2 symmetric stretching or tension); a small band at 1544 cm^{-1} (C=C stretching/vibration of benzene rings); two medium peaks at 1493 cm^{-1} and 1452 cm^{-1} (C=C stretching/vibration of benzene rings); a very small peak at 1028 cm^{-1} (C=C stretching/vibration of benzene rings); a medium peak at 749 cm^{-1} (out-of-plane C–H bending of benzene rings), indicating one substituent in it, which is the linear aliphatic chain of its structure; and a large narrow peak at 696 cm^{-1} (an out of plane benzene-ring bending vibration) (Miller and Coates, 2025, Wu and Criddle, 2021).

The larvae-treated EPS IR spectrum showed the same band pattern as the control, with few specific changes described as follows. Only one change localized at the analytical region, which was an increased intensity of the peak at 2917 cm^{-1}, corresponding to C–H stretching vibrations of methylene groups (-CH2) (Miller and Coates, 2025); and two changes localized at the fingerprint region, one of them an appearance of peaks at 1102 cm^{-1} and 1027 cm^{-1}, related with carbonyl and hydroxyl groups (Miller and Coates, 2025); and the second one an increased intensity and broadening of the region between 906 cm^{-1} and 1037 cm^{-1}, associated with aromatic C–H bending in plane vibrations (Al-Kadhemy et al., 2016; Miller and Coates, 2025).

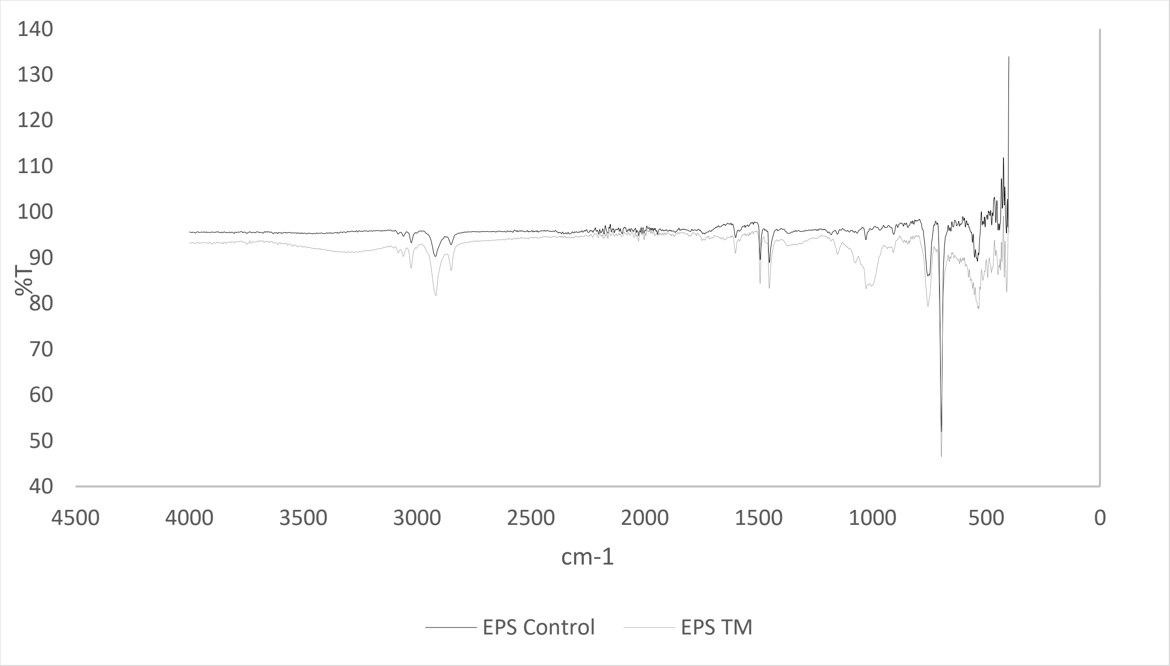

Figure 3. FTIR-ATR analysis of EPS. Control EPS (EPS Control, black line) and EPS with *T.molitor* larvae and maize (EPS TM, gray line).

3.3 FTIR analysis of frass

The IR spectrum from the control frass (Figure 4) exhibited the following bands: A low intensity but broad peak at 2918 cm^{-1}, associated with C-H stretch of alkanes; a low intensity narrow peak at 2851 cm^{-1}, associated with C-H stretch of sp3 carbons of alkanes; an also broad peak from 1510 to 1724 cm^{-1}, with maximum at 1646 cm^{-1}, usually associated with a -C=C stretch of alkenes or C=O stretch of ketones; and a broad peak from 974 to 1160 cm^{-1}, with maximum at 1027 cm^{-1}, usually associated with C-C stretch of alkanes or C-O stretch from an alcohol group (Miller and Coates, 2025).

The frass from EPS-fed larvae presented the same band pattern as the control, with the only difference being a marked increase of intensity of the narrow peak at 696 cm^{-1}, associated with a C-H bend, which was consistently found among all the spectra of the frass of the EPS-fed larvae (Figure 4).

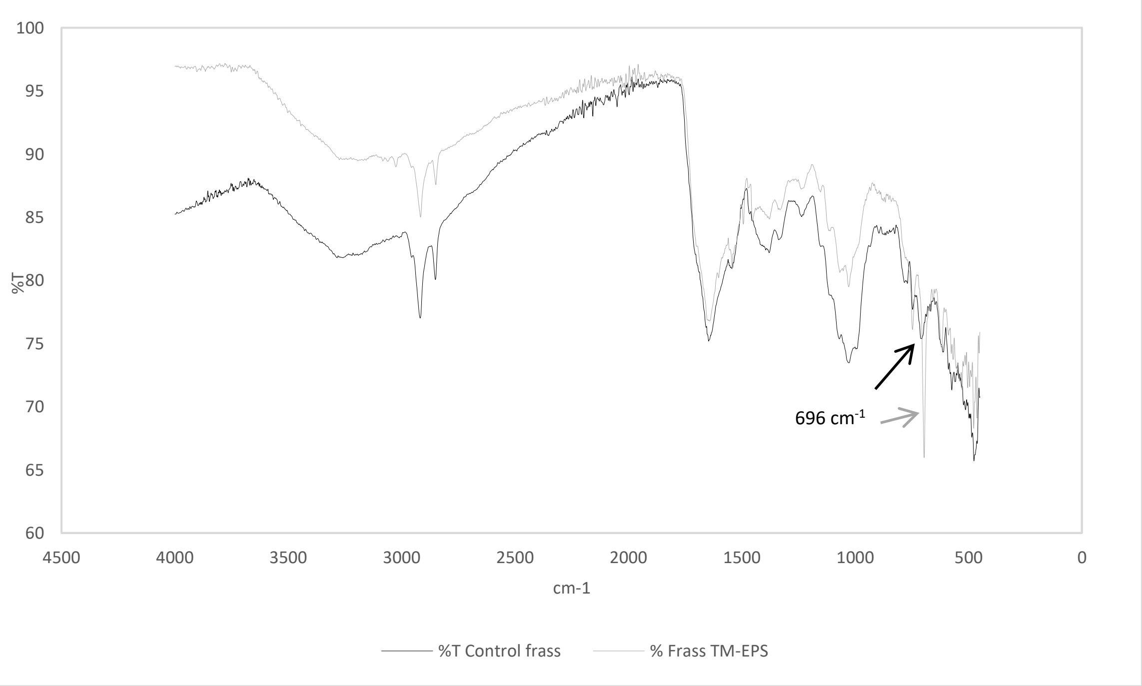

Figure 4. FTIR-ATR of the frass of *T.molitor*. Control frass of larvae fed only with maize (black line), and frass of the larvae fed with both EPS and maize grains (gray line).

3.4 Exuviae FTIR-ATR analysis

The FTIR analysis of the control exuvia was conducted as follows: A very broad band from 3120 cm^{-1} to 3369 cm^{-1} with maximum peak at 3272 cm^{-1}, corresponding to vibrations involving stretching of -OH of chitin (Mwita et al., 2024; Vargas-Venegas et al., 2024); a narrow large peak at 2915 cm^{-1}, which is characteristic of stretching vibration of -CH$_2$ bond of -CH$_2$OH (Marin-Morales et al., 2025; Mwita et al., 2024; Vargas-Venegas et al., 2024); a narrow large peak at 2849 cm^{-1} corresponding to C−H stretching of the polysaccharide of chitin (Marin-Morales et al., 2025); a broad medium band at 1622 cm^{-1} indicating a specific hydrogen bond of C=O with the hydroxyl-methyl group of the next chitin residue of the same chain (Marin-Morales et al., 2025; Vargas-Venegas et al., 2024); a series of sharp medium-intensity bands between 1560 cm^{-1} and 1476 cm^{-1} with maximum peak at 1542 cm^{-1}, indicating C=O stretch and amide groups (N−H bending and

C−N stretching) (Marin-Morales et al., 2025; Mwita et al., 2024); a unique medium narrow peak at 1470 cm^{-1}, which indicates C-H bend of methyl groups (Marin-Morales et al., 2025); a small broad band at 1379 cm^{-1}, suggesting amide II (N-H bending) and amide III (C-N stretching) groups (Marin-Morales et al., 2025; Vargas-Venegas et al., 2024); a broad medium band from 1160 to 1300 cm^{-1} with maximum peak at 1231 cm^{-1}, which indicates amide II (N-H bending) and amide III (C-N stretching) groups (Marin-Morales et al., 2025); and a small narrow peak at 1028 cm^{-1} and a medium narrow peak at 1068 cm^{-1}, which indicate C-O-C and C-O-H stretching in the glucosamine ring of chitin, respectively (Marin-Morales et al., 2025; Vargas-Venegas et al., 2024).

As for the exuviae from EPS-fed larvae, FTIR analysis was performed on seven samples. All of them displayed the chitin/protein pattern presented in the control treatment, but three of these presented distinct particularities, described below and presented in Figure 5: A small sharp peak at 1700 cm^{-1} may indicate aryl-conjugated species (see Discussion section); a broad medium band with maximum at 1175 cm^{-1}, indicative of C-O-C and C-O-H bonds; and a broadening of the band from 961 to 1047 cm^{-1} with maximum peak at 1027 cm^{-1}, consistent with hydrogen bonding or ionic environment (Marin-Morales et al., 2025; Miller and Coates, 2025)

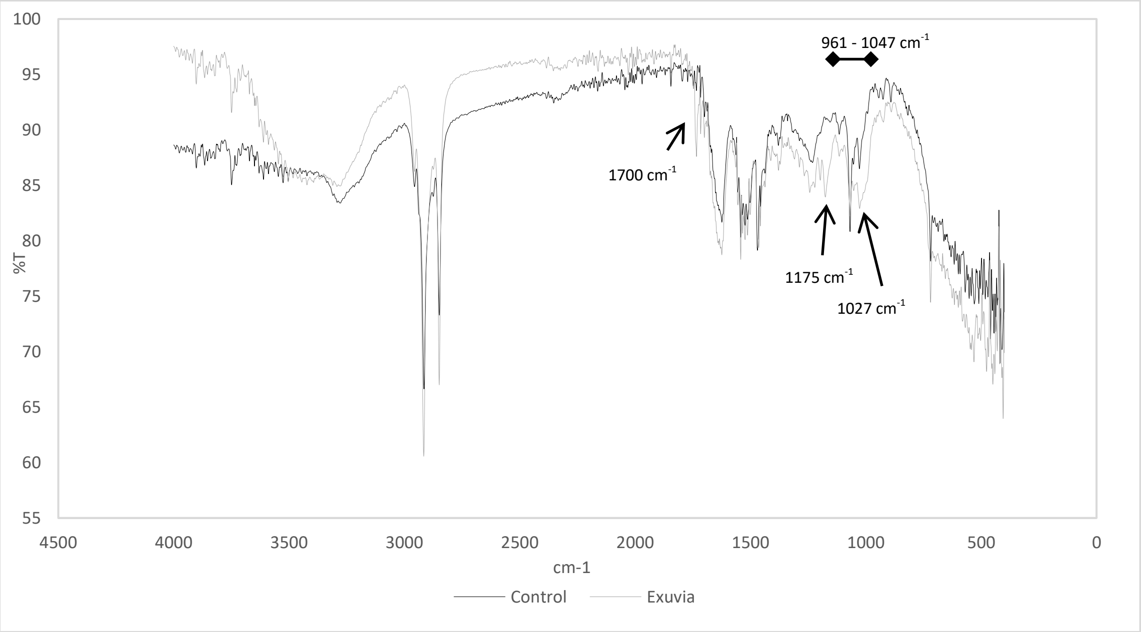

Figure 5. FTIR-ATR of the exuviae of *T.molitor*. Control exuvia of larvae fed only with maize grains (black line); and exuvia of the larvae fed with EPS and maize grains (gray line).

4. DISCUSSION

4.1 EPS consumption rates

The consumption of EPS by larvae of *T. molitor* under different conditions has been extensively documented internationally (Yang et al, 2018; Jiang et al., 2021; Tsochatzis et al., 2021; Yang et al., 2021; Bulak et al. 2021); however, there is scarce research in Mexico about the consumption of these plastics with native *T. molitor* larvae (Rodríguez-Carreón et al., 2021). Therefore, we aimed to study the consumption rates of larvae from Tuxtla Gutierrez, Chiapas, México, and their response when they are co-fed with also native maize grains.

In the experiment without maize, the slight slope from week 1 to 3 (phase 1) reflects a latency phase during which the larvae barely consume plastic, likely because they are metabolically preparing to utilize it. Afterwards, a sudden steeper slope from week 4 to 5 (phase 2) indicates a bingeing episode within a short period of time (two weeks), representing an increase in the consumption rate of the EPS. In a third phase, the larvae return to a moderate consumption rate, from week 6 to week 9, indicated by a level slope. From week 10 to 11, after adding 3 g of maize, a new bingeing behavior was developed, which indicates a second phase of increased consumption of the plastic, promoted by co-feeding the larvae with maize grains, but of less intensity than the first bingeing episode from week 4 to 5, indicative of its capacity to alternate between bingeing episodes by co-feeding with grains, but of less intensity. The totality of the EPS wasn't consumed, as 75.8 % (2.274 g) remained after the 11th week of the experiment.

In the experiment with EPS and maize, the almost flat slope between weeks 1 and 2 (phase 1) might indicate an adaptation phase in which the larvae are adjusting their microbiome and/or metabolism to the new feeding conditions, to then have a very pronounced bingeing episode for the next two weeks (phase 2), increasing significantly its feeding rate. The slope of this phase is steeper than that from the same phase of the experiment without maize, demonstrating that co-feeding with maize grains enhances the larvae capacity to consume EPS, consistent with other reports with different grains (Yang et al, 2018; Yang et al 2020; Peng et al, 2020; Tsochatzis et al., 2021; Chávez Muñoz et al., 2022; Machona et al., 2022). The third phase showed a decrease in its feeding rate from week 5 to week 11, similar to the treatment without maize, and it was maintained throughout the rest of the experiment. It is noteworthy that by week 9, when all the original maize grains had been fully consumed and an additional 3 g of maize were added, no change in the slope of the plastic consumption rate by the larvae was observed, which suggests that, despite the addition of more maize grains, the larvae were unable to initiate a new bingeing episode, as was the case of the previous experiment without maize, possibly because they had already adapted to co-metabolize EPS and maize from the beginning of the experiment. Control experiments without larvae showed no weight loss throughout the whole experiment in both the absence and presence of EPS, ruling out substrate or environmental factors as contributors to EPS weight loss.

According to Yang et al (2018), the latency phase represents the development of physiological adaptations of the larvae to enhance their ability to process and degrade plastics. Ding et al. (2024) extends this by offering three possible explanations: an adaptation of the gut microbiome composition, a response to the oxidative stress triggered by the plastic, or an increased enzymatic production which would facilitate the breakdown of the plastic polymer. The second phase of bingeing could depend on the age of the larvae or their degree of starvation, as seen in larvae of *Culex tarsalis* Coquillett, *Aedes aegypti* L. and *Anopheles albimanus* Wiedemann (Rashed and

Mulla, 1989). This second phase warrants further study, as Dhammi et al. (2022) proposed that an increase in the feeding rate in caterpillars of *Chloridea viscerens* could be explained as a mechanism of tolerance against toxic molecules, which could be one of the explanations behind the increase in the feeding rate of *T. molitor*'s larvae in the bingeing phases of both experiments, namely, as a mechanism of tolerance against the toxic molecules of the EPS, rather than feeding from it. At the third phase, the EPS consumption rate stabilizes to a more level slope. It cannot be ruled out that both the latency and the third level phase could reflect loss of interest for the plastic, as the simplest explanation is usually the most plausible.

A probable explanation for the stimulatory effect of maize grains on consumption rate may lie in the composition of the maize pericarp. The pericarp is made of lignin, a polysaccharide backbone made of arabinoxylans and ferulic acid, which is a hydroxycinnamic acid that comprises a phenol group in its structure (Chateigner-Boutin et al., 2016). Ferulic acid is the most abundant phenolic acid in cereals, typically accounting for 60 to 90% of total phenolic acids in grains such as wheat, barley, maize, rice, among others (Horvat et al., 2020), and maize pericarp has been reported to contain high levels of ferulic acid (Chateigner-Boutin et al., 2016). Also, ferulic acid is known to function as a bridge that cross-links lignin and the arabinoxylan backbone in the cell wall of some grasses through ether and ester bonds (Chateigner-Boutin et al., 2016). On the other side, plastics like polystyrene, polyurethane and polyethylene terephthalate have chemical structures comprised of benzene groups, ether and ester bonds (Ma et al., 2024; Jiang et al., 2024), suggesting that co-feeding larvae with food rich in compounds with these molecular characteristics will prompt the proliferation of the appropriate microbiome or the expression of the proper enzymatic machinery in their guts necessary to digest such plastics. In *T. molitor*, such enzymes have been reported to be esterases, chitinases and phosphatases, among others (Przemieniecki et al., 2020). This could explain that, at a molecular level, the consumption of plastic is greater when larvae are co-fed with grains than when they aren't, but this needs further experimentation for confirmation.

4.2 FTIR-ATR analysis of EPS

FTIR-ATR analysis was employed to identify consistent and discernible spectral differences between control samples and those obtained from larvae fed with EPS and maize grains (Figure 3). The infrared spectra of expanded polystyrene samples from both the control and *T. molitor* experiments were consistent over the 5-week period. Notable differences between the control EPS and the EPS-larvae treated samples were found, and are described as follows: an increased intensity of the peak at 2917 cm^{-1} in the analytical region, corresponding to C–H stretching vibrations of methylene groups ($-CH_2$) (Miller and Coates, 2025), which suggests an increased concentration of aliphatic $-CH_2-$ groups probably as a result from surface accumulation of EPS hydrocarbon fragments derived from partial oxidation of the aliphatic hydrocarbon chains by activity of the larvae on the polystyrene.

The rest of the changes in the EPS from larvae were observed at the fingerprint region, which were also consistent over the 5-week period. Even minor changes in this region may reflect variations in molecular conformation, folding, or chemical environment, when compared with the control EPS (Kasem et al, 2023; Bec et al., 2019). The appearance of peaks at 1102 cm^{-1} and 1027 cm^{-1} in the larvae EPS could indicate the formation of new chemical species or oxidative reactions, consistent with previous studies reporting the formation of carbonyl and hydroxyl groups after biological degradation (Parthasarathy et al., 2022) or after photodegradation (Pushpadass et al., 2010). Furthermore, the region between 906 and 1037 cm^{-1}, associated with aromatic C–H bending in plane vibrations (Al-Kadhemy et al., 2016), showed increased intensity and broadening, indicating potential structural modifications in the aromatic rings (Miller and Coates, 2025).

These spectral changes align with recent findings that *T. molitor* larvae can induce measurable chemical modifications in EPS, potentially through the action of gut microbiota, leading to partial degradation and surface oxidation (Reish et al., 2024).

4.3 FTIR-ATR analysis of frass

The frass from *T. molitor* larvae was analyzed by FTIR-ATR in the presence and absence of EPS over the 5 week experiment, during which all the measurements from the control EPS and EPS-larvae experiments showed a remarkable similar band pattern (Figure 4). Although the molecular composition of the frass of *T. molitor* has been poorly reported, an old work from Weaver et al. from 1990 mentioned the presence of butyric, propionic and valeric acid in the frass of late-instar larvae of *T. molitor*, which could in part explain the bands encountered in our analysis, but it cannot be ruled out the presence of more components.

Our study showed minor differences between the FTIR spectra of the control frass and the frass from the EPS-fed larvae, although all the measurements had a consistent band pattern over the 5 week experiment. The only marked difference among the frass of the control and from the EPS-fed larvae was the marked increase of intensity of the narrow peak at 696 cm^{-1}, associated with a C-H bend, which was consistently found among all the spectra of the frass of the EPS-fed larvae. The consistent finding of this increased peak at 696 cm^{-1} in all the frass from the EPS-fed larvae, which coincides with the narrow aromatic peak at the same wavenumber from the control EPS (Figure 3), may indicate mechanical fragmentation and excretion of polystyrene-derived phenyl moieties that pass through the gut of the larvae and end up in the feces, without detectable ring oxidation (Miller and Coates, 2025). A similar peak is also seen in the FTIR spectra of the frass from PS-fed superworms and of greater wax moths from Jiang et al. (2021), possibly also indicating their inability to degrade the benzene ring. Nevertheless, it was absent from the frass of the EPS-fed *T. molitor* of the same authors, which according to them indicated destruction of the benzene ring. This difference may be attributed to variations in diet composition, gut microbiota, larval stage, or EPS particle size. Regardless, the results suggest the release of EPS subproducts derived from its degradation in the frass of the larvae.

4.4 FTIR-ATR analysis of exuviae

FTIR-ATR spectra of exuviae from larvae in contact with EPS and from control larvae over the 5 week of exposure with the plastic were analyzed to identify spectral differences that could be attributable to EPS degradation products (Figure 5). Both groups of larvae were fed with maize grains. Band assignments were made based on previously published spectral data by different authors.

T. molitor exuviae is made primarily of carbohydrates (~49.2 %), protein (~26.3 %), dietary fiber (~22.8%), crude fat (~3.7%), ash (5.4%) and moisture (~6.4%); of these, chitin makes up for 10-25%. This composition is reflected in the FTIR measurements done in this and in other works (Marín-Morales et al., 2025; Kwon et al, 2020).

Although apparent spectral differences were observed between the exuviae from the control and EPS groups, a deeper analysis indicates that plastic derived by-products are not deposited in the exuviae, as it is detailed below. A sharp peak at 1700 cm^{-1} appeared in only 3 of the 7 EPS-exposed exuviae, which could hint at aryl-conjugated species accumulated in its exuviae; yet, its sporadic appearance suggests it may not be a cuticular component and could simply reflect surface accumulation (Miller and Coates, 2025). Additional evidence is needed, so it was not considered further in this work . Likewise, a broad medium band with maximum peak at 1175 cm^{-1} appeared in the EPS-exposed exuvia, in contrast with the control experiment; this feature could be due to surface-adsorbed, EPS-derived, oxygenated species generated during an oxidative fragmentation of the polystyrene, but it does not distinguish surface deposition from metabolism incorporation (Miller and Coates, 2025). Finally, a broadening of the band from 961 to 1047 cm^{-1} with maximum peak at 1027 cm^{-1}, is consistent with hydrogen bonding or ionic environment, but not necessarily represents a new functional group (Miller and Coates, 2025). The work of Zarzosa and Kobayashi (2024) showed that chitin obtained from larvae of *Zophoba morio* fed with polystyrene showed similar FTIR band positions and widths compared to controls, suggesting that simple ingestion and incorporation do not significantly alter the bands. Also, Ilijin et al. (2024) found no significant differences between exuviae of *T. molitor* fed with wheat bran alone and those fed with wheat bran and PS. This aligns with our observation of minimal differences between exuviae of control and EPS groups, despite using maize instead of bran; nonetheless, those minimal differences could be attributed to maize co-feeding. Additional studies are required to elucidate the metabolic fate of the polymer, but it surpasses the objectives of this work.

5. Conclusion

A behavior pattern for the consumption of EPS by the larvae was determined in this study, characterized by an initial slow lag phase with a duration between 2 to 3 week, where the larvae is barely consuming the plastic, suggesting an adaptation prior to its consumption; followed by a bingeing phase where the consumption of the plastic is increased drastically; to then stabilize into a delayed consumption phase. We encountered that, after this delayed consumption phase, supplying maize grains could induce a new bingeing phase.

Consistent spectral distinctions in plastic and frass samples distinguished the control and the EPS groups, suggesting chemical changes in the plastic due to larval action, and the presence of plastic-derived compounds in the frass, respectively. The exuviae of the larvae, when analyzed in depth, showed no consistent spectral distinctions between control and the EPS-samples, indicating neither metabolic incorporation nor surface deposition of plastic-derived compounds, so further evidence is needed. These results also demonstrate that a FTIR-ATR analysis is sufficient for a rapid detection of consistent presence or absence of changes in spectral measurements of EPS without the need of sample pre-treatment.

Using living organisms for bioremediation is a sustainable alternative, and the larvae of *T. molitor* is suitable for it. Its feasibility for production and its incorporation as poultry feed make it an economically attractive alternative. Studying the behavior of these larvae could help in the development of technologies for entomoremediation of plastics and to improve the acquisition of knowledge from other species of larvae that also feed on plastic that could be used also for bioremediation.

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Conflict of interest

The authors have no conflict of interest to declare.

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