Biodegradation of polyethylene, biodegradable-polyethylene bags and corn residues using Tenebrio molitor larvae

Biodegradación de polietileno, bolsas de polietileno biodegradable y residuos de maíz mediante larvas de Tenebrio molitor

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RESUMEN

Se estudió la capacidad de degradación de distintos tipos de plástico a partir de su ingestión por gusanos de la harina (larvas de Tenebrio molitor), así como su supervivencia. Se utilizaron bolsas construidas con plástico denominado polietileno de baja densidad (LDPE); plástico compostable, fabricado a partir de residuos de maíz (CPE); y plástico de polietileno con tecnología d2w (d2wPE).

Las larvas de T. molitor fueron sometidas a un periodo de inanición de 7 días y luego alimentadas únicamente con los distintos tipos de plásticos durante 56 días. Se prepararon dos grupos de muestras con los diferentes plásticos. Por un lado, los plásticos se expusieron a un pretratamiento de fotodegradación UV de 260 a 285 nm y luego se utilizaron como única fuente de alimento para las larvas. Por otro lado, se realizó el mismo experimento sin el pretratamiento UV. El objetivo era comparar los posibles efectos de la radiación UV sobre los plásticos y su posterior degradación por las larvas.

Las tasas máximas de degradación con pretratamiento UV fueron del 98%, 77% y 4% para las muestras de CPE, LDPE y d2wPE, respectivamente. Las tasas de degradación sin pretratamiento fueron del 79%, 54% y 3%, y se obtuvieron en el mismo orden. En ambos escenarios, la supervivencia de las larvas sólo se vio afectada significativamente en las muestras de d2wPE, alcanzando una tasa media de mortalidad del 98%. Para el resto de las muestras, la tasa media de mortalidad fue sólo del 12%.

Palabras clave: Larvas de Tenebrio molitor, biodegradación, LDPE, CPE, d2wPE, fotodegradación

ABSTRACT

The degradation capacity of different types of plastic from its ingestion by mealworms (the larvae of *Tenebrio molitor*), as well as its survival, was studied. Bags build from plastic nominally known as low-density polyethylene (LDPE); compostableplastic, made from corn waste (CPE); and polyethylene with d2w technology (d2wPE) plastic, were used.

T. molitor larvae were subjected to a 7-day starvation period and then only fed with the different types of plastics for 56 days. Two groups of samples were prepared with the different plastics. On the one hand, the plastics were exposed to a UV photodegradation pretreatment from 260 to 285 nm and then used as the only source of food for the larvae. On the other hand, the same experiment was carried out without the UV pretreatment. The objective was to compare the possible effects of UV radiation on the plastics and their subsequent degradation by the larvae.

The maximum degradation rates with UV pretreatment were 98%, 77% and 4% for CPE, LDPE, and d2wPE samples, respectively. Degradation rates without pretreatment were 79%, 54%, and 3%, and were obtained in the same order. In both scenarios, larval survival was significantly affected just for the d2wPE samples, reaching an average mortality rate of 98%. For the rest of the samples, the average mortality rate was only 12%.

Keywords: *Tenebrio molitor* larvae, biodegradation, LDPE, CPE, d2wPE, photodegradation

1 INTRODUCTION

Given the main characteristics of plastic: durability, high mechanical strength and cost-effectiveness, products made with this material have become of great importance in the development of human life (Geyer *et al*., 2017). There are various types of plastics that meet multiple needs of society, such as containers, electronic devices, household implements, footwear.

However, when their life cycle ends, they are transported and accumulated in both aquatic and terrestrial environments, causing negative impacts, such as obstruction of rain ways, contamination of water bodies, death of wildlife and their infiltration into food chains (Chae and An, 2018; Schwarz *et al*., 2019).

Wrapping plastic bags are commonly made of polyethylene (PE) or polypropylene (PP). Polyethylene can be classified into different categories, depending on molecular density or branching. In the elaboration of plastic bags, high (HDPE) and low density (LDPE) stand out. Both materials are not biodegradable, so their decomposition can take several centuries (Müller *et al*., 2012).

D2w plastic bags, known as oxo-biodegradables, are also composed of PE or PP, and oxo-additives are added to stimulate degradation and allow microorganisms to convert the polymer into CO_2 and biomass (Ojeda *et al*., 2009). The incorporation of additives produces new plastics that, if exposed to moisture or sunlight, fragment into very fine pieces. Fragmentation time is unpredictable depending on these abiotic factors (Abdelmoez *et al*., 2021). Following fragmentation, plastic can become seemingly invisible, but persists in the environment in the form of microplastics (Musioł *et al*., 2017). Controversy has been generated about the biodegradability of oxoplastics, since once fragmented, not enough low molecular weight plastic is produced that microorganisms can degrade (Portillo *et al*., 2016). Although the biodegradation of oxoplastics is widely documented and emphasizes the ability of microorganisms to consume their fragments, it has been discussed that biodegradation rates are variable under different controlled experimental parameters, which in turn differ from environmental ones (Montazer *et al*., 2019). The literature discusses various biodegradation rates ranging from 5 to 60% (Thomas *et al*., 2014).

The so-called bioplastics are those that use renewable resources as raw materials for their elaboration, for example, starch extracted from corn (Sarasa *et al*., 2009, Riera, 2020). They are manufactured on a small scale and are usually more expensive than conventional plastics, therefore, their use is not massive and applications are limited to products with an important ecological or high value marketing, such as some medicinal products (Cortez Suarez *et al*., 2022). A biodegradable plastic or bioplastic is not always compostable. Compostable plastics are those that biodegrade rapidly during the composting process (without altering the value of the compost), while transforming into water, inorganic compounds, carbon dioxide and biomass, leaving no distinguishable or toxic residue. The difference between biodegradable polymers and compostable polymers lies in the rate of biodegradation, its disintegration and toxicity (Niaounakis, 2013).

Plastics are not biodegraded completely (Abdelmoez *et al*., 2021), so plastic pollution is virtually irreversible. Potential impacts of this pollution are changes in carbon and nutrient cycles, changes in soil, sediment and aquatic ecosystems, biological impacts on endangered species, ecotoxicity, and related social impacts (Musioł *et al.* , 2017) . For example, when plastic waste reaches bodies of water, indigenous fauna can feed on it, mistaking it for food. Long-term exposure to this diet can cause carcinogenic, hematological, and cytogenetic effects (Thompson *et al.* , 2009) .

The mealworm (*Tenebrio molitor*) has been shown to be able to feed on and degrade plastic once it transits through its intestines (Brandon *et al*., 2018; Yang *et al*., 2015a). In a study by Brandon *et al*. (2018) degradation efficiencies of 73% were observed in a 32-day experimental period for LDPE samples, using 120 larvae of *T. molitor*, and an initial mass of 1.8 g of sample. Using polyurethane samples, Bulak *et al*. (2021) found a degradation efficiency of 47% after 58 days of experimentation, with 500 larvae of *T. molitor*, and 2.6 g of polyurethane as the main diet of the larvae.

The gut microbial communities of larvae can change when the diet is exclusively plastic (Wang *et al*., 2022). However, intestinal bacteria are known to be responsible for the degradation of this material (Liu *et al*., 2022; Przemieniecki *et al*., 2020; Yang *et al*., 2015b), some of the main ones being proteobacteria, bacteroides and firmicutes species.

On the other hand, it is possible to significantly degrade many materials through their exposure to ultraviolet (UV) radiation. For example, when polystyrene is subjected to ultraviolet radiation in the presence of air, it experiences rapid yellowing and gradual embrittlement (Yousif and Haddad, 2013). In addition, photodegradation is used to simulate the exposure of plastics to meteors (Öborn *et al*., 2022).

In this work, the ability of *Tenebrio molitor larvae* to degrade different types of plastic through their ingestion was investigated, and their survival was reported. LDPE, CPE and d2wPE samples were used.

2 MATERIALS AND METHODS

Acquisition of tenebrios and adaptation

The larvae of *T. molitor* were acquired at a local veterinary center, where they were fed wheat bran and vegetables. On average, the length of the larvae was 1.8 cm, and their mass was 76 mg. In the lab, the larvae underwent a 72-hour fasting period before starting the experimental diet based exclusively on plastic, as suggested by Wang *et al.* (2022).

Plastics selection and pre-treatment

Three types of plastics were selected in bag presentation with high availability in the market: LDPE, d2wPE and CPE bags. For standardized handling, 0.1 g samples of each type of plastic were taken and pre-treated for photodegradation using UV light with a wavelength range of 260 to 285 nm. This UV pretreatment was used hoping to obtain a plastic material of greater degradability for the larvae of *T. molitor*. Simultaneously, another identical group of samples was prepared, which were not subjected to UV pretreatment.

Assembly of the experiment

Two experiments were run simultaneously. On the one hand, three glass containers were used, one for each type of plastic with UV pretreatment. In each, 0.1 g of plastic and 20 randomly selected *T. molitor* larvae were placed. On the other hand, the same events were mounted using the different types of plastic without UV pretreatment. The vessels were kept at room temperature with controlled humidity in dark conditions. Each experiment was conducted in duplicate and controls with larvae fed on wheat bran were considered to quantify larval mortality and the possible toxic effects of plastic consumption.

Determination of degradation

The experimental process was carried out over a period of 56 days. A cut was made every 7 days, in which the survival of *T. molitor was recorded*, as well as the mass of each type of plastic by gravimetric method.

To determine the survival of *T. molitor*, a count of live and dead larvae was carried out in each cut. The dead larvae were removed from experimentation and replaced with new larvae, constantly keeping 20 larvae alive in each container.

For the determination of degradation, the excrement residues were removed from the larvae and the samples of each type of plastic were placed in a desiccator for 12 hours to remove moisture. Subsequently, its mass was recorded using an analytical balance and the mass lost in each cut was determined. The plastic frames were reintegrated into each experiment with their respective larvae after each determination.

Statistical analysis

Statistical analysis was performed using Minitab software using ANOVA analysis of variance.

3 RESULTS AND DISCUSSION

Photodegradation with UV light

After constant exposure of the samples to UV radiation over a period of 7 days, no significant changes in the mass of the samples were observed. However, some samples showed physical changes. The CPE samples took on a yellowish coloration and gained hardness, becoming more fragile when handled. As for the d2wPE samples, they also took a yellowish coloration, gained hardness, and rolled up. LDPE samples showed no apparent change.

Degradation capacity of plastics

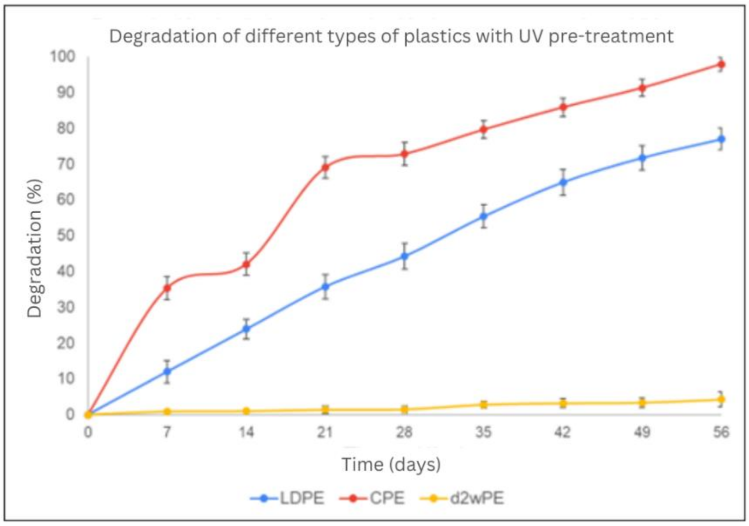
The degradability of plastics was assessed by feeding *T. molitor* larvae with fragments of LDPE, d2WPE and CPE bags over a period of 56 days.

The larvae demonstrated the ability to degrade LDPE and CPE samples, with and without UV pretreatment. However, no favorable results were observed in the samples of d2wPE as they were not consumed by the larvae in any of the scenarios, leading to the death phase and a cannibalism effect among them.

Degradation with UV pretreatment

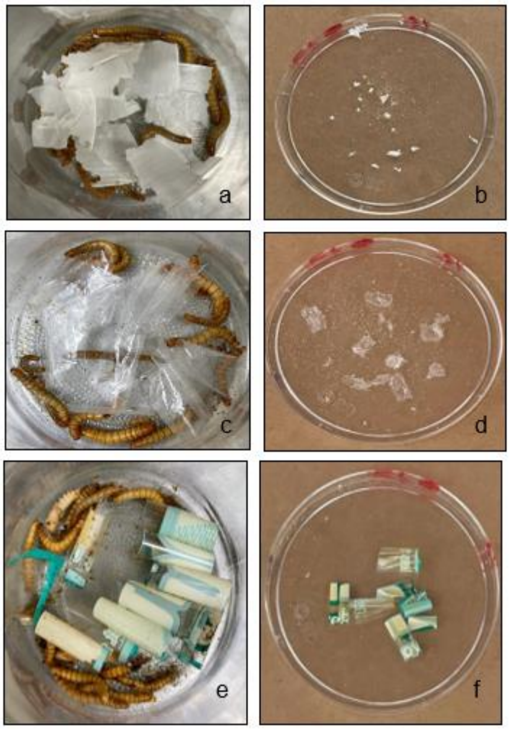
As shown in Figure 1, after the starvation period, *T. molitor* larvae managed to degrade up to 35% of the CPE sample in the first 7 days. It is possible that this is due to the nature of this plastic's composition, its main component are residues of corn, which, in turn, are part of the regular diet of the tenebrios. In the case of LDPE samples, 12% degradation was observed in the same period. In contrast to these two plastics, no significant change in degradation was present in the d2wPE samples. The maximum degradation was 98% for CPE (56 days), followed by LDPE (77% at the end of the experimentation). The lowest degradation was obtained with d2wPE, of 4%.

Figure 1. Comparison of the degradation capacity of LDPE, CPE and d2wPE with UV pretreatment. Source: own creation



The degradation of the different plastics with pretreatment at 7 and 56 days of experimentation can be observed in Figure 2.

Figure 2. Degradation of CPE, LDPE, d2wPE with UV pretreatment after 7 days of experimentation (a, c and e). In a and c, the bites of the larvae began to be visible. In case e, only the effects of change of shape and color in the sample are appreciated. Degradation of CPE, LDPE, d2wPE with UV pretreatment at 56 days of experimentation (b, d and f). In b and d, the mass loss of the samples was almost total, otherwise in f, where there is no apparent degradation. Source: own creation



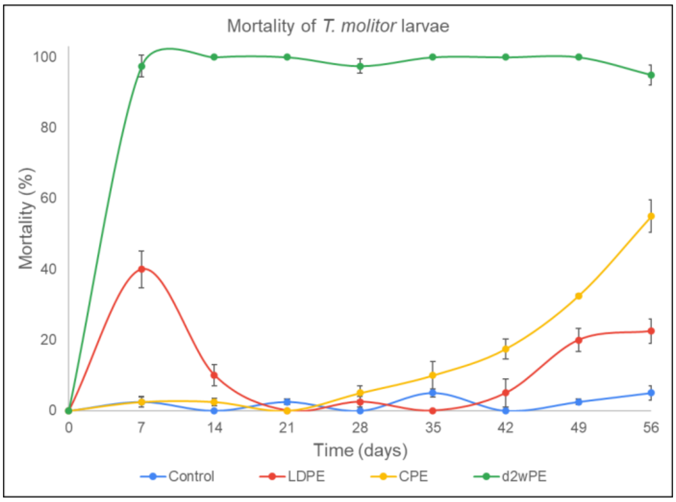
Mortality with UV pretreatment

Due to the low consumption of d2wPE, there was a larval mortality of 97% in the first 7 days, reaching 100% in each section by the end of the experimentation (Figure 3). The trend in larval mortality could be attributed to the inks and chemicals present on the surface of the bag. The lack of a substrate other than d2wPE led to a behavior of cannibalism among the larvae.

In the case of LDPE, there was a high mortality (40%) after 7 days, possibly due to the period of adaptation of the larvae to this new diet. However, after 14 days a stabilization stage with a low mortality rate (2.5 - 10%) began. After 42 days, due to the low availability of food, mortality increased again, reaching 22% and leading to the cannibalistic behavior, previously described.

With the CPE, the lowest mortality was presented compared to the other samples, maintaining a rate of 0 - 2.5% after 28 days of experimentation. From this time, the scarcity of food caused an exponential increase in mortality to reach 55%, the same time in which 98% of plastic degradation was reached.

Figure 3. Mortality of *T. molitor* larvae fed wheat bran (control), LDPE, CPE and d2wPE with UV pretreatment. Source: own creation

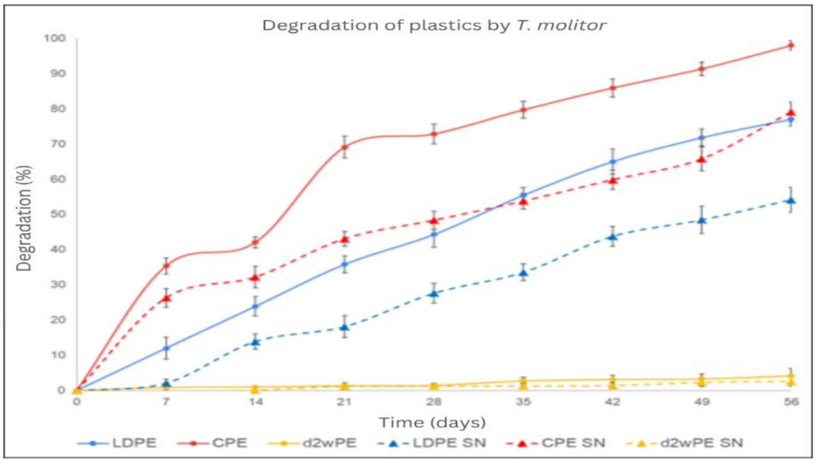


Degradation without UV pretreatment

In the tests carried out without UV pretreatment, a similar behavior was observed in the degradation trend of all samples, although in lower percentages, this occurred because there were no alterations in the physical properties of the plastic, making it more complex to consume for the larvae.

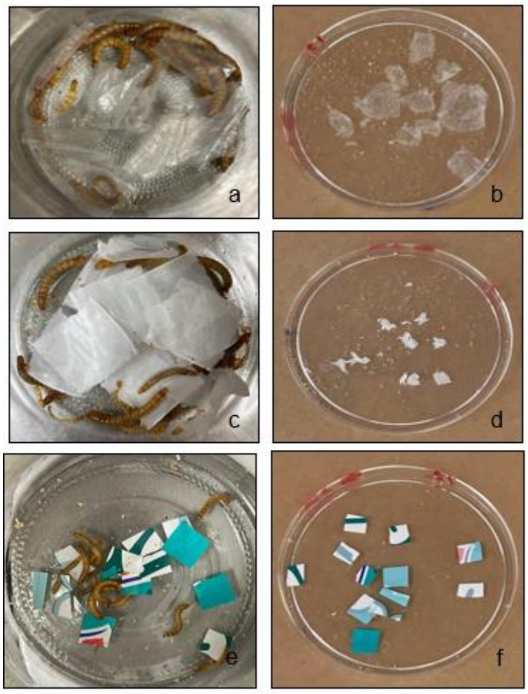
As shown in Figure 4, for LDPE, the degradation rate was approximately 23% lower compared to tests performed under UV treatment, peaking at 54% at the end of the experimentation. For CPE, a 20% lower degradation rate was observed than that with UV pretreatment. The maximum rate of degradation was 79%. In the case of tests with d2wPE, the same percentage of degradation was obtained with and without UV pretreatment, reaching only a maximum rate of 4%.

Figure 4. Comparison of the degradation capacity of LDPE, CPE and d2wPE with and without (SN) UV pretreatment (SN). Source: own creation



In Figure 5 the degradation of the different plastics without pretreatment at 7 and 56 days of experimentation can be appreciated.

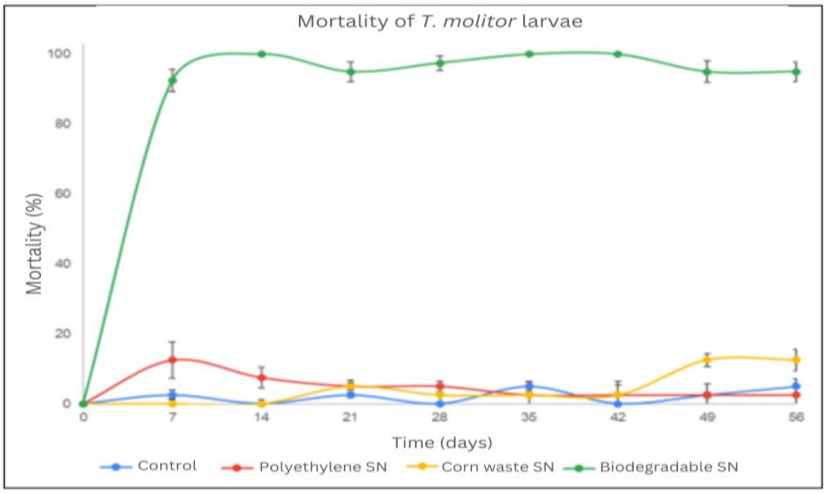
Figure 5. Degradation of CPE, LDPE, d2wPE without UV pretreatment after 7 days of experimentation (a, c and e). Note that in cases a and c, the bites of the larvae began to be visible. Degradation of CPE, LDPE, d2wPE without UV pretreatment at 56 days of experimentation (b, d and f). In b and d, the mass loss of the samples exceeded 60% of the degradation, compared to the f sample, where there is no apparent degradation. Source: own creation



Mortality without UV pretreatment

As can be seen in Figure 6, with the exception of d2wPE, in no sample larval mortality exceeded 13%, since the plastic-bags samples were available to the larvae throughout the experimental period, this also applies to the fact that there were no cannibalism behaviors and the mortality rate remained similar to the control, dominating the survival of larvae.

Figure 6. Mortality of *T. molitor* larvae fed wheat bran (control), LDPE, CPE and d2wPE without UV (SN) pretreatment. Source: own creation



4 CONCLUSIONS

T. molitor larvae can degrade LDPE and CPE with and without UV photodegradation pretreatment. The maximum percentages of degradation obtained were 77% and 98%, as well as 54% and 79%, respectively. The use of UV pretreatment favored the degradation of PE probably reducing its molecular weight and altering its mechanical properties, making it more accessible as larvae feed. However, with respect to d2wPE, it was observed that its physicochemical properties prevented the effects of degradation by UV radiation. Given this, d2wPE did not turn out to be an attractive or accessible food for the larvae, so there were high mortality rates and cannibalism behavior.

Consumption of LDPE and CPE had no apparent harmful effects on *T. molitor* larvae. By employing UV pretreatment, the degradation process was accelerated making the plastic food more accessible to the larvae, so it was quickly exhausted and competition for it was generated.

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