Abstract: *Uloomoides dermestoides* are used as a broad-spectrum medical insect in the alternative treatment of various diseases. Preliminary volatilome studies carried out to date have shown, as the main components, methyl-1,4-benzoquinone, ethyl-1,4-benzoquinone, 1-tridecene, 1-pentadecene, and limonene. This work focused on the production of metabolites and their metabolic variations in *U. dermestoides* under stress conditions to provide additional valuable information to help better understand the broad-spectrum medical uses. To this end, VOCs were characterized by HS-SPME with PEG and CAR/PDMS fibers, and the first reported insect essential oils were obtained. In HS-SMPE, we found 17 terpenes, six quinones, five alkenes, and four aromatic compounds; in the essential oils, 53 terpenes, 54 carboxylic acids and derivatives, three alkenes, 12 alkenes (1-Pentadecene, EOT1: 77.6% and EOT2: 57.9%), 28 alkanes, nine alkyl disulfides, three aromatic compounds, 19 alcohols, three quinones, and 12 aldehydes were identified. Between both study approaches, a total of 171 secondary metabolites were identified with no previous report for *U. dermestoides*. A considerable number of the identified metabolites showed previous studies of the activity of pharmacological interest. Therefore, considering the wide variety of activities reported for these metabolites, this work allows a broader vision of the therapeutic potential of *U. dermestoides* in traditional medicine.

**Keywords:** *Uloomoides dermestoides*; VOCs; HS-SPME; PBET; essential oils; GC-MS; insect

1. Introduction

The tenebrionid *Uloomoides dermestoides* (Fairmaire, 1983) (Coleoptera: Tenebrionidae) (synonyms: Alphitobius; Dermestoides; Martianus dermestoides; Palembus dermestoides) is a darkling beetle endemic to the Indomalaya and Papua regions [1]. It is used as a broad-spectrum medical insect in the alternative treatment of various diseases, such as bronchial asthma, dermatitis, rheumatoid arthritis, hemorrhoids, inflammation and pain in the liver and kidneys, Parkinson’s disease, diabetes mellitus, HIV, and different types of cancer [2–5]. Studies carried out to date have been preliminary, detecting methyl-1,4-benzoquinone (MBQ), ethyl-1,4-benzoquinone (EBQ), 1-pentadecene, and limonene as the significant volatile organic components (VOCs) that are expelled by the insect in its defense secretions [6,7]. To date, just a few investigations have evaluated the pharmacological activity of organic extracts derived from *U. dermestoides*. Among the biological activities evaluated in these investigations are anti-inflammatory [3], cytotoxic [4], antiproliferative [8], antidiabetic [5], antioxidant and antimicrobial activity [9] without attributing the biological activity found to a specific metabolite. The metabolites reported for *U. dermestoides* do not explain the wide spectrum of the medicinal use of the insect; therefore,
a more extensive study of the metabolomics of the insect and its variation due to stimuli is required to explain the wide spectrum of entopharmacological use.

Currently, secondary metabolites of insects are obtained using various methodologies, of which we can highlight organic extraction [10,11], solid-phase extraction (SPE) [12,13], and headspace-solid phase microextraction (HS-SPME) [14,15], in conjunction with gas chromatography (GC) coupled to a flame ionization detector (FID) and/or mass spectrometry (MS) for the identification of metabolites. For *U. dermestoides*, only the VOCs have been evaluated by HS-SPME, with the polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber [6]. However, it has several limitations due to its inability to capture nonpolar and highly polar compounds; therefore, this fiber does not cover a broad spectrum of compounds. For this reason, it remains to be determined whether *U. dermestoides* can produce other chemically diverse secondary metabolites.

Moreover, like other organisms such as plants and bacteria, *U. dermestoides* would be expected to modify its metabolism if the conditions change, such as when the insect has been ingested. In this sense, the production of *U. dermestoides* metabolites and their metabolic variations under stress conditions would provide additional valuable information to help better understand the broad spectrum of its medical uses. In this work, the number of fibers with different polarities used in HS-SPME was increased to determine a higher number of the compounds present in the volatilome of *U. dermestoides* and the metabolic changes that occurred when the insect was subjected to a stimulus that emulated their consumption. The results led to the procurement of the first essential oils from insects, and their characterization allows us to understand that the metabolomics of the insect is more complex than previously reported, thus justifying the wide spectrum of medicinal uses attributed to *U. dermestoides*.

2. Results

2.1. VOCs Collection with CAR/PDMS Fiber

To determine greater amounts of VOCs of *U. dermestoides*, the volatilome profiles were analyzed by GC-MS under two stimulus conditions over time with CAR/PDMS and PEG fibers. The compounds identified in treatment 1 (T1) in the initial 5 min showed EBQ as the major component (39.21%), followed by limonene (21.82%), and then MBQ, p-benzoquinone (BQ), α-pinene, and 1-pentadecene with 14.29%, 6.11%, 4.35%, and 4.33%, respectively, of the total. However, over time, the relative percentages of these compounds changed—the relative amounts of EBQ and MBQ gradually decreased to as low as 7.44% and 1.94%, respectively, at 18 h, while the relative amounts of limonene, α-pinene, and 1-pentadecene more than doubled during the same period (Table 1, representative chromatograms: Figures S1 and S3, see Supplementary Materials).

Adding treatment 2 (T2) to the insects had a notable effect on the metabolite profile starting in the first minutes of exposure (Table 1). Here, at 5 min of incubation, 1-pentadecene was the major compound present, comprising 53.74% of the total, followed by limonene (29.22%), and α-pinene, 1-tridecene, EBQ, and carene, with 8.95%, 2.63%, 1.9%, and 1.15% of the total, respectively. Over time, the relative amount of 1-pentadecene decreased to as low as 22.44% of the total at 18 h of incubation. The concentrations of the rest of these VOCs tended to increase over time, with limonene making up 47.7% of the total and α-pinene, 1-tridecene, EBQ, and carene making up, respectively, 11.85%, 7.1%, 6.71%, and 3.9% of the total at 18 h of exposure. An increase in the type of VOCs released was in fact observed at 1 h of incubation with T2. This increase was particularly important for sesquiterpene compounds.

Most importantly, when the two tested stimuli were compared, the beetles that were subjected to T1 immediately released quinone derivatives and limonene, and their metabolism increased the amounts of 1-pentadecene and terpenes when in the presence of the simulated gastric fluid. These differences were also observed at different incubation points, e.g., at 18 h, when higher concentrations of terpenes such as limonene and pinene were observed for insects subjected to T1.
Table 1. VOCs produced by *U. dermestoides* and collected with CAR/PDMS fiber.

| No. | Compounds                        | KI Ref | Exp | 5 min T1 | T2 | 1 h T1 | T2 | 6 h T1 | T2 | 18 h T1 | T2 | 24 h T1 | T2 |
|-----|----------------------------------|-------|-----|---------|-----|--------|-----|--------|-----|--------|-----|--------|-----|
|     |                                  |       |     |         |     |        |     |        |     |        |     |        |     |
| 1   | p-Benzquinone                    | 912   | 920 | 6.11    | 2.9 | 0.83   |     |         |     |        |     |        |     |
| 2   | Methyl-1,4-benzoquinone          | 1015  | 1016| 14.3    | 0.07| 13.6   | 0.95| 3.59   | 0.68| 1.94   | 1.42| 12.1   | 0.97|
| 3   | Ethyl-1,4-benzoquinone           | 1215  | 1112| 39.2    | 1.9 | 33.3   | 4.89| 9.9    | 2.65| 7.44   | 6.71| 34.1   | 2.56|
| 4   | Hydroquinone                     | 1241  | 1291| 0.67    | 0.11| 0.18   |     |         |     |        |     |        |     |
| 5   | 2-Methylhydroquinone             | 1378  | 1359| 0.4     | 0.37| 0.11   | 0.13| 0.13   | 0.09| 0.21   |     |        |     |
| 6   | 2-Ethylhydroquinone              | 1413  | 1440| 1.16    | 0.98| 0.46   |     |         |     | 0.18   | 0.13| 0.65   | 0.65|

**Quinones**

**Terpenes**

**Alkenes**

**Aromatic compounds**

RT: Retention time, KI: Kovats index, T1: Agitated only insects, T2: digested with PBET solution insects.

2.2. VOCs Collection with PEG Fiber

As shown in Table 2 (representative chromatograms: Figures S2 and S4), when the extraction was performed with PEG fiber on beetles under T1, the most prevalent polar compounds identified at the initial time point were quinones. Here, EBQ was the most abundant quinone, making up 54.25% of the total VOCs, followed by MBQ, EHQ, HQ, BQ, and MHQ, which made up, respectively, 17%, 7.45%, 3.54%, 3.08%, and 1.49%; in addition to quinones, 1-pentadecene and limonene were observed in somewhat significant amounts, making up 5.64% and 1%, respectively, of the total. From the initial time point up to 24 h of assessment, the concentrations of the quinones EBQ, EHQ, and MHQ increased to 58.25%, 12.49%, and 2.96% of the total, respectively, while the levels of 1-pentadecene, HQ, BQ, and limonene compounds decreased. Likewise, at 6 h and 18 h, other monoterpenes such as cis-verbenol, verbenone, myrtenol, and perillol were observed, as well as two phenol-type compounds, namely m-cresol and 3,4-dimethylphenol.

Moreover, when the extraction was performed with PEG fibers for beetles under T2, 1-pentadecene (67.62%) was initially the main compound observed, followed by EBQ (11.02%), limonene (10.21%), 1-tridecene (1.94%), MBQ (1.16%), and MHQ (0.464%). As the incubation time was increased, these percentages changed considerably; for example, at 24 h, the concentrations of 1-pentadecene and 1-tridecene decreased considerably and those of the quinone-derived compounds increased two- to three-fold from the initial
values. Unlike the results in T1, when the sample was treated with T2, neither cis-verbenol, verbenone, and myrtenol monoterpenes nor phenol-like metabolites were detected.

Table 2. VOCs produced by U. dermestoides and collected with PEG fiber.

| No. | Compounds                      | KI  | Exp | 5 min T1 | 5 min T2 | 1 h T1 | 1 h T2 | 6 h T1 | 6 h T2 | 18 h T1 | 18 h T2 | 24 h T1 | 24 h T2 |
|-----|-------------------------------|-----|-----|----------|----------|--------|--------|--------|--------|--------|--------|--------|--------|
| 1   | 2-Benzquinone                 | 912 | 920 | 3.08     | 0.11     | 0.62   |        |        |        |        |        | 1.29   |        |
| 2   | Methyl-1,4-benzoquinone       | 1015| 1016| 17       | 1.16     | 15.7   | 1.46   | 10.8   | 1.09   | 7.42   | 1.69   | 17.1   | 2.61   |
| 3   | Ethyl-1,4-benzoquinone        | 1215| 1112| 54.3     | 11       | 54.7   | 14.7   | 49     | 16.9   | 49.8   | 22.4   | 58.5   | 26.9   |
| 4   | Hydroquinone                  | 1241| 1291| 3.54     | 0.13     | 0.94   | 0.21   | 0.43   | 2.34   |        |        |        |
| 5   | 2-Methylhydroquinone          | 1378| 1359| 1.49     | 0.46     | 2.76   | 0.61   | 2.28   | 1.2    | 2.55   | 2.03   | 2.96   | 1.47   |
| 6   | 2-Ethylhydroquinone           | 1413| 1440| 7.45     | 4.89     | 12.1   | 7.74   | 15.8   | 12.3   | 21.6   | 22.4   | 12.5   | 16.4   |
|     |                               |     |     |          |          |        |        |        |        |        |        |        |        |
| 1   | α-Pinene                      | 922 | 936 | 0.54     | 0.5      | 0.29   |        |        |        |        |        | 0.66   |        |
| 3   | Carene                        | 1008| 1001| 0.19     | 0.15     | 0.07   |        |        |        |        |        | 0.17   |        |
| 6   | Limonene                      | 1020| 1033| 1        | 10.2     | 1.18   | 10.5   | 2.98   | 10.2   | 0.64   | 9.26   | 0.54   | 13.6   |
| 13  | cis-Verbenol                  | 1148| 1158| 0.32     | 0.19     |        |        |        |        | 0.17   |        |        |
| 14  | p-Cymen-8-ol                  | 1172| 1191| 0.11     | 0.3      | 0.16   |        |        |        |        |        |        |
| 15  | Verbenone                     | 1204| 1204| 0.35     | 0.28     |        |        |        |        |        |        |        |
| 16  | Myrtenol                      | 1213| 1212| 0.06     |          |        |        |        |        |        |        |        |
| 17  | Perillol                      | 1297| 1318| 0.1      |          |        |        |        |        |        |        |        |
| 9   | Di-epi-α-cedrene-(I)          | 1414| 1414|          |          |        |        |        |        |        |        |        |
| 12  | Cuparene                      | 1502| 1540| 0.66     | 0.03     | 0.4    | 0.13   | 0.04   |        |        |        |        |
|     |                               |     |     |          |          |        |        |        |        |        |        |        |        |
| 3   | m-Cresol                      | 1053| 1088|          |          | 0.25   | 2.6    |        |        |        |        |        |
| 4   | 3,4-Dimethylphenol            | 1167| 1180|          |          | 0.2    | 2.11   |        |        |        |        |        |
| 1   | 2,2’-Bifuran                  | 1334| 1335| 0.09     |          |        |        |        |        |        |        |        |
|     |                               |     |     |          |          |        |        |        |        |        |        |        |
| 1   | 1-Tridecane                   | 1287| 1295| 1.94     | 0.5      | 1.37   | 0.45   | 0.31   | 0.47   |        |        |        |
| 4   | 1,14-Pentadecadiene           | 1480| 1479| 3.06     | 1.04     | 0.59   | 0.59   | 1.14   | 0.88   | 0.74   |        |        |
| 5   | 1-Pentadecene                 | 1486| 1494| 5.64     | 67.6     | 10.2   | 62     | 14.5   | 58.3   | 10.5   | 42.2   | 2.16   | 38.2   |

In general terms, U. dermestoides under the four analysis conditions produced the same types of molecules: quinones, terpenes, and aliphatic alkenes. Moreover, as reported previously, methyl-1,4-benzoquinone, ethyl-1,4-benzoquinone, limonene, 1-tridecene, and 1-pentadecene were present under all four conditions. On the other hand, other secondary metabolites were identified that had not been previously reported for U. dermestoides. These results suggested the need to obtain essential oils under both stimulus conditions to obtain a broader vision of the metabolome of U. dermestoides.

2.3. Obtention and Characterization of Essential Oils

During sample processing by hydrodistillation, characteristic behaviors of each essential oil were observed, namely the striking color of the distillation water for EOT1 and the absence of this in EOT2. On the other hand, oil drops were visible only in EOT2. The yield of the essential oils EOT1 and EOT2 was 0.19% and 0.9%, respectively. For EOT1, 61 compounds were identified, which corresponds to 93.70%; meanwhile, 87 compounds, which represented 92.98% of the total were identified in EOT2. In both oils, the major component was 1-pentadecene, comprising 77.6% and 57.9% of the total in EOT1 and EOT2, respectively, followed by 1-tridecene (3%), limonene (2.9%), pentacosane (1.8), and tricosane (1.5%), in EOT1, while hentriacontane (6.53%), palmitic acid (6.47%), linoleic acid (2.79%), tricosane (2.79%), pentacosane (2.2%), 1-tridecene (1.75%), oleic acid (1.72%) and limonene (1.43%) were the most abundant compounds in EOT2 (Table 3, Figures S5 and S6).
Table 3. Identified compounds of *U. dermestoides* essential oils.

| No. | Compounds                        | EOUd1 |         | EOUd2 |         | KI Ref |
|-----|----------------------------------|-------|---------|-------|---------|-------|
|     |                                  | RA (%)| KI Exp  | RA (%)| KI Exp  |       |
| 1   | α-Pinene                         | 0.239 | 0.106   | 0.239 | 0.106   | 922   |
| 18  | β-thujene                        | 0.015 | 0.050   | 0.015 | 0.050   | 968   |
| 19  | Isolimonene                      | 0.008 | 0.005   | 0.008 | 0.005   | 974   |
| 3   | 2-Carene                         | 0.074 | 0.037   | 0.074 | 0.037   | 996   |
| 4   | α-Phellandrene                   | 0.002 | 0.000   | 0.002 | 0.000   | 997   |
| 20  | α-Terpine                        | 0.037 | 0.084   | 0.037 | 0.084   | 1008  |
| 5   | α-Cymene                         | 0.029 | 0.017   | 0.029 | 0.017   | 1025  |
| 6   | D-Limonene                       | 2.898 | 1.435   | 2.898 | 1.435   | 1033  |
| 8   | p-Cymenene                       | 0.006 | 0.004   | 0.006 | 0.004   | 1081  |
| 21  | Terpinen-4-ol                    | 0.002 | 0.005   | 0.002 | 0.005   | 1024  |
| 22  | β-Cedrene                        | 0.012 | 0.005   | 0.012 | 0.005   | 1423  |
| 23  | cis-Thujopsene                   | 0.005 | 0.002   | 0.005 | 0.002   | 1435  |
| 12  | Cuparene                         | 0.004 | 0.005   | 0.004 | 0.005   | 1502  |
| 24  | Phytan                           | 0.026 | 0.017   | 0.026 | 0.017   | 1811  |
| 25  | Squalene                         | 0.045 | 0.020   | 0.045 | 0.020   | 2847  |
| 26  | 28-Nor-17β(H)-hopane             | 0.395 | 0.038   | 0.395 | 0.038   | 3393  |
| 27  | 22R-17α(H),21β(H)-bishomohopane  | 0.097 | 0.020   | 0.097 | 0.020   | 3313  |
| 28  | γ-Sitosterol                      | 0.248 | 0.128   | 0.248 | 0.128   | 3351  |

Table continued...
| No. | Compounds                          | EOUd1 RA (%) | EOUd1 KI | EOUd2 RA (%) | EOUd2 KI | KI Ref |
|-----|------------------------------------|--------------|----------|--------------|----------|--------|
| 6   | Decene                             | 82.78%       | 61.51%   | 82.78%       | 61.51%   | 985    |
| 7   | Dodecene                           | 0.006±0.001  | 1190.8   | 0.011±0.000  | 1190.1   | 1187   |
| 1   | 1-Undecene                         | 3.020±0.126  | 1295     | 1.755±0.006  | 1293.4   | 1287   |
| 3   | 1-Tetradecene                      | 0.434±0.019  | 1392.2   | 0.231±0.002  | 1391.6   | 1385   |
| 4   | 1,14-Octadecadiene                 | 0.456±0.056  | 1479.2   | 0.714±0.007  | 1477.4   | 1480   |
| 5   | 1-Pentadecene                      | 77.671±0.906 | 1517     | 57.965±0.240 | 1507.1   | 1486   |
| 8   | 1-Hexadecene                       | 0.278±0.017  | 1592.6   | 0.183±0.006  | 1591.3   | 1587   |
| 9   | Alkenes                            |              |          |              |          |        |
| 10  | Heptadecadiene                     |              |          |              |          |        |
| 11  | Heptadecene                        |              |          |              |          |        |
| 12  | Pentacosene                        |              |          |              |          |        |
| 12  | Alkyl disulphides                  | 0.02%        | 1.16%    |              |          |        |
| 1   | Methyl n-butyl disulfide           |              |          |              |          |        |
| 2   | Ethyl n-butyl disulfide            |              |          |              |          |        |
| 3   | Propyl n-butyl disulfide           |              |          |              |          |        |
| 4   | Methyl n-heptyl disulfide          | 0.007±0.002  | 1269     | 0.206±0.001  | 1268.6   | 1268.6 |
| 5   | Ethyl n-heptyl disulfide           | 0.003±0.001  | 1360.4   | 0.028±0.003  | 1359.5   | 1359.5 |
| 6   | Propyl n-heptyl disulfide          | 0.002±0.001  | 1425.6   | 0.057±0.002  | 1424.7   | 1424.7 |
| 7   | Butyl n-heptyl disulfide           |              |          |              |          |        |
| 8   | Pentyl n-heptyl disulfide          |              |          |              |          |        |
| 9   | Diheptyl disulfide                 | 0.007±0.001  | 1738.5   | 0.776±0.003  | 1738.2   | 1738.2 |
| 12  | Aldehydes                          | 0.001%       | 0.18%    |              |          |        |
| 1   | Phenylacetaldehyde                 |              |          |              |          |        |
| 2   | Hexadeconal                        |              |          |              |          |        |
| 12  | Alcohols                           |              |          |              |          |        |
| 1   | 1-Heptanol                          | 0.003±0.001  | 1090     | 0.007±0.001  | 1089.4   | 1092   |
| 12  | Quinones                           | 0.00%        |          |              |          |        |
| 1   | Ethyl-1,4-benzoquinone             |              |          |              |          |        |
| 2   | 2-Ethylhydroquinone                |              |          |              |          |        |
| 12  | Carboxylic acids and derivatives   | 0.58%        |          | 13.12%       |          |        |
| 1   | 2,4-Dimethyl-5-hexanolide          | 0.002±0.000  | 1181.6   | 0.020±0.001  | 1180.1   | 1180.1 |
| 2   | Dodecanoic acid                    |              |          |              |          |        |
| 3   | n-Hexyl salicylate                 |              |          |              |          |        |
| 4   | Myristic acid                      |              |          |              |          |        |
| 5   | Ethyl myristate                    |              |          |              |          |        |
| 6   | Methyl palmitate                   | 0.021±0.001  | 1929     | 0.033±0.001  | 1928.5   | 1927   |
| 7   | Pentadecanoic acid                 |              |          |              |          |        |
| 8   | Palmitic acid                      | 0.219±0.031  | 1967.6   | 6.475±0.160  | 1982.2   | 1964   |
| 9   | Ethyl palmitate                    | 0.022±0.002  | 1996.5   | 0.212±0.002  | 1996.2   | 1982   |
| 10  | Linolenic acid                     |              |          |              |          |        |
| 11  | γ-Palmolactone                     |              |          |              |          |        |
| 12  | Linoleic acid                      |              |          |              |          |        |
| 13  | Oleic acid                         |              |          |              |          |        |
| 14  | Ethyl-9,12-octadecadienoate        | 0.066±0.008  | 2165.9   | 0.345±0.019  | 2164.2   | 2164.2 |
| 15  | Ethyl oleate                       | 0.015±0.001  | 2170.9   | 0.364±0.069  | 2170.9   | 2149   |
| 16  | Stearic acid                       | 0.077±0.004  | 2175     | 0.511±0.013  | 2173.5   | 2179   |
| 17  | Ethyl stearate                     |              |          |              |          |        |
| 18  | Stearyl acetate                    | 0.156±0.017  | 2213.2   | 0.119±0.002  | 2211.3   | 2211   |
| 12  | Aromatic compounds                 | 0.00%        |          |              |          |        |
| 5   | Benzothiazole                      |              |          |              |          |        |
| 6   | 6-tert-Butyl-3-Methylanisole       |              |          |              |          |        |
In the essential oils, 11 terpenes, 12 carboxylic acids and their derivatives, 17 alkanes, eight alkenes, nine alkyl disulfides, and three aromatic compounds were identified for the first time in this report. Among the metabolites exclusively found in EOT1, were the terpenes β-thujene and phytan, as well as the alkanes heneicosane and 1-cyclohexyleicosane. On the other hand, squalene, n-hexyl salicylate, ethyl myristate, pentadecanoic acid, linoleic acid, γ-palmitolactone, ethyl stearate, 5-ethyldecane, 6-methylundecano, 3-ethyltetradecanoic acid, trioctanate, pentacosane, benzothiazole, 6-tert-butyl-3-methylanisole, methyl n-butyl disulfide, ethyl n-butyl disulfide, propyl n-butyl disulfide, butyl n-heptyl disulfide, and pentyl n-heptyl disulfide were found exclusively in EOT2.

Due to the presence of carboxylic acids, alcohols, and aldehydes in essential oils, it was necessary to confirm these results by derivatization. For this analysis, 38 and 77 compounds were identified, which corresponds to an increase of 3.75% and 6.38% in their identification in EOT1 and EOT2, respectively. Therefore, new compounds—25 terpenes, 33 carboxylic acids, and 18 alcohols—were identified with derivatization by silanization (Table 4).

Table 4. Identified compounds of U. dermestoides essential oils derivatized by silanization.

| No. | Compounds                               | EOUd1       | EOUd2       | KI Ref |
|-----|----------------------------------------|-------------|-------------|-------|
|     | Carboxylic acids and derivatives        | RA (%)      | RA (%)      |       |
| 19  | Butanoic acid                          | 0.60%       | 2.71%       |       |
| 20  | Valeric acid                           | 0.04 ± 0.005| 871.5       | 891   |
| 21  | Peracetic acid                         | 0.010 ± 0.000| 982.4       | 975   |
| 22  | Lactic acid                            | 0.030 ± 0.007| 1006        |       |
| 23  | Caproic acid                           | 0.058 ± 0.001| 1070.3      | 1057  |
| 24  | 2-Ethylhexanoic acid                   | 0.002 ± 0.001| 1076        | 1071  |
| 25  | Heptanoic acid                         | 0.016 ± 0.000| 1168.4      | 1166  |
| 26  | Benzoic acid                           | 0.030 ± 0.001| 1184.9      | 1186  |
| 27  | 2-Octanoic acid                        | 0.089 ± 0.001| 1247        | 1232  |
| 28  | Succinic acid                          | 0.007 ± 0.001| 1255        | 1313.2|
| 29  | Propionylglycine                       | 0.006 ± 0.001| 1366.2      | 1384  |
| 30  | Nonanoic acid                          | 0.009 ± 0.001| 1386.4      | 1386  |
| 31  | Decanoic acid                          | 0.019 ± 0.004| 1528        | 1526  |
| 32  | m-Hydroxybenzoic acid                  | 0.009 ± 0.001| 1545.2      | 1542  |
| 33  | 10-Undecenoic acid                     | 0.007 ± 0.001| 1546.4      | 1559  |
| 34  | Pimelic acid                           | 0.003 ± 0.001| 1614.2      | 1628  |
| 35  | Suberic acid                           | 0.012 ± 0.004| 1710.3      | 1689  |
| 36  | Tridecanoic acid                       | 0.008 ± 0.001| 1755.3      | 1748  |
| 37  | Azelaic acid                           | 0.061 ± 0.019| 1806.9      | 1787  |
| 38  | β-Resorcylic acid                      | 0.005 ± 0.002| 1833.9      | 1822  |
| 39  | 9-Tetradecenoic acid                   | 0.018 ± 0.003| 1841.4      |       |
| 40  | Tetradecanoic acid                     | 0.231 ± 0.004| 1854.1      | 1845  |
| 41  | Sebacic acid                           | 0.005 ± 0.006| 1907        | 1920  |
| 42  | Pentadecanoic acid                     | 0.004 ± 0.001| 1925.1      | 1942  |
| 43  | 13-Methyltetradec-9-enoic acid         | 0.000 ± 0.001| 1946.9      |       |
| 44  | 9-Hexadecenoic acid                    | 0.002 ± 0.001| 1974.5      | 1977  |
| 45  | cis-9-Hexadecenoic acid                | 0.005 ± 0.001| 2023.7      | 2017  |
| 46  | cis-10-Heptadecenoic acid              | 0.002 ± 0.001| 2127.5      | 2126  |
| 47  | Margaric acid                          | 0.041 ± 0.006| 2152.6      | 2140  |
| 48  | cis-11,14-Eicosadienoic acid           | 0.010 ± 0.000| 2414.8      | 2413.2|
| 49  | cis-11-Eicosenoic acid                 | 0.014 ± 0.001| 2420.2      | 2419.7|
| 50  | Arachidic acid                         | 0.039 ± 0.002| 2447        | 2437  |
| 51  | 1-Monopalmitin                         | 0.007 ± 0.001| 2608.9      | 2606  |
| 52  | Docosanoic acid                        | 0.016 ± 0.002| 2645        | 2638  |
| 53  | Triacontadienoic acid                  | 0.033 ± 0.005| 3433.1      |       |
| 54  | Dotriacontadienoic acid                | 0.025 ± 0.005| 3639.9      |       |
| No. | Compounds                                      | EOUd1 RA (%) | KI Exp | EOUd2 RA (%) | KI Exp | KI Ref |
|-----|-----------------------------------------------|--------------|--------|--------------|--------|--------|
| 2   | 2,2-Dimethyl-3-pentanol                       | 0.18%        |        |              |        |        |
| 3   | Furfuryl alcohol                              | 0.102 ± 0.017|        | 1003.8       |        |        |
| 4   | 2,4-Dimethyl-3-pentanol                       | 0.011 ± 0.000| 1009.8 |              |        | 975.3  |
| 5   | 3-heptanol                                    | 0.016 ± 0.001| 1018.7 | 0.095 ± 0.010| 1018.7 |        |
| 6   | 2-heptanol                                    | 0.034 ± 0.014| 1025.2 | 0.394 ± 0.018| 1024.8 |        |
| 7   | 2,3-Butanediol                                |              |        | 0.282 ± 0.006| 1044   | 1040   |
| 1   | 1-Heptanol                                    |              |        | 0.004 ± 0.001| 1088.4 | 1090   |
| 8   | 3-Ethylphenol                                 |              |        | 0.001 ± 0.001| 1223.1 | 1220   |
| 9   | 4-hydroxybenzenemethanol                      |              |        | 0.003 ± 0.001| 1520.3 | 1500   |
| 10  | 1-Dodecanol                                   |              |        | 0.046 ± 0.004| 1574.5 | 1575   |
| 11  | 1-Tetradecanol                                |              |        | 0.039 ± 0.002| 1768.5 | 1768   |
| 12  | 1-Pentadecanol                                |              |        | 0.011 ± 0.000| 1879.4 | 1846   |
| 13  | 2-Pentadecanol                                |              |        | 0.011 ± 0.001| 1879.4 | 1877   |
| 14  | 1-Hexadecanol                                 |              |        | 0.028 ± 0.003| 1966.4 | 1966   |
| 15  | 1-Heptadecanol                                |              |        | 0.013 ± 0.000| 2069.5 | 2065   |
| 16  | Oleyl alcohol                                  |              |        | 0.017 ± 0.003| 2136.3 | 2126   |
| 17  | 1-Hexacosanol                                  |              |        | 0.007 ± 0.001| 2949   | 2950   |
| 18  | 1-Octacosanol                                  |              |        | 0.016 ± 0.004| 3149   | 3148   |
| 19  | 1-Dotriacontanol                               |              |        | 0.019 ± 0.004| 3532.9 | 3529.9 |

| No. | Compounds                                      | EOUd1 RA (%) | KI Exp | EOUd2 RA (%) | KI Exp | KI Ref |
|-----|-----------------------------------------------|--------------|--------|--------------|--------|--------|
| 29  | Myrtenoic acid                                | 0.014 ± 0.000| 1535.4 |              |        |        |
| 30  | 18-Norabieta-8,11,13-triene                   | 0.004 ± 0.001| 1978.2 |              |        |        |
| 31  | 10,18-Bisnorabieta-8,11,13-triene              | 0.014 ± 0.002| 2040.9 |              |        |        |
| 32  | Allopregnane                                  | 0.009 ± 0.003| 2204.8 |              |        | 2175   |
| 33  | Levopimic acid                                | 0.012 ± 0.001| 2262.7 |              |        | 2246.6 |
| 34  | Pimaric acid                                  | 0.020 ± 0.004| 2281.7 |              |        | 2287   |
| 35  | 7-Ethyl-1,4a,7-trimethyl-3,4,4b,5,6,8,10,10a-octahydro-2H-phenanthrene-1-carboxylic acid | 0.015 ± 0.004| 2293.3 |              |        |        |
| 36  | 15-Isobutyl-(13α-H)-isocopalane                | 0.110 ± 0.001| 2294.2 |              |        |        |
| 37  | Isopimaric acid                               | 0.010 ± 0.002| 2337.1 |              |        | 2329   |
| 38  | 8-Pimarenic acid                              | 0.102 ± 0.004| 2353.8 |              |        |        |
| 39  | Abiet-8-en-18-oic acid                        | 0.179 ± 0.003| 2371.8 |              |        |        |
| 40  | Dehydroabietic acid                           | 0.451 ± 0.011| 2391.7 |              |        | 2385   |
| 41  | 12α-Hydroxy-5α-pregnane                       | 0.007 ± 0.003| 2756   |              |        |        |
| 42  | Coprostone                                    | 0.010 ± 0.002| 2835.6 |              |        | 2822   |
| 43  | 17α,21β-28,30-Bisnorhopane                    | 0.177 ± 0.010| 2873.4 | 0.005 ± 0.000| 2858.8 |        |
| 44  | Gammacerane                                   | 0.674 ± 0.031| 3135.1 | 0.019 ± 0.004| 3122.8 |        |
| 45  | Cholesterol                                   | 0.053 ± 0.002| 3151.5 |              |        | 3143   |
| 46  | Germanicol                                     | 0.012 ± 0.001| 3208.6 |              |        |        |
| 47  | 3-Epirotenol                                   | 0.182 ± 0.014| 3244.3 |              |        |        |
| 48  | Campesterol                                   | 0.010 ± 0.001| 3259   |              |        | 3220   |
| 49  | Stigmastereol                                  | 0.196 ± 0.014| 3291   | 0.090 ± 0.003| 3274.3 |        |
| 50  | β-Sitosterol                                   | 0.991 ± 0.014| 3349.8 |              |        | 3348   |
| 51  | Fucosterol                                    | 0.286 ± 0.004| 3366.3 |              |        |        |
| 52  | Avenaster                                     | 0.015 ± 0.001| 3421.7 |              |        |        |
| 53  | 24-Methylenecycloartenol                      | 0.07 ± 0.009 | 3459.4 | 0.042 ± 0.004| 3460   |        |

| No. | Compounds                                      | EOUd1 RA (%) | KI Exp | EOUd2 RA (%) | KI Exp | KI Ref |
|-----|-----------------------------------------------|--------------|--------|--------------|--------|--------|
| 7   | 2,4-Dihydroxyacetophenone                     | 0.001 ± 0.001| 1726.1 | 0.007 ± 0.001| 1726.4 | 1709.3 |

In this analysis, the metabolites found only in EOT1 were 15-isobutyl-(13α-H)-isocopalane, 2-octanoic acid, suberic acid, benzenepropanoic acid, and 2,4-dimethyl-3-pentanol. In the case of EOT2, terpenes as myrtenoic acid, 18-norabieta-8,11,13-triene, 10,18-bisnorabieta-8,11,13-
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triene, allopregnane, pimaric acid, 7-ethyl-1,4a,7-trimethyl-3,4,4b,5,6,8,10,10a-octahydro-2H-
phenanthrene-1-carboxylic acid, isopimaric acid, 8-pimarenic acid, abiet-8-en-18-oic acid, 12x-
hydroxy-5α-pregnane, coprostone, cholesterol, germanicol, 3-epimoretenol, campesterol, and
avenasterol were found; carboxylic acid and derivatives: butanoic acid, valeric acid, peracetic,
10-undecenoic acid, tridecanoic acid, 2-resorcylic acid, 9-tetradecenoic acid, 9-
hexadecenoic acid, cis-10-heptadecenoic acid, cis-11,14-eicosadienoic acid, cis-11-eicosenoic
acid, archadicular, 1-monopalmotinin, docosanoic acid, triacontadioneic acid and dotria-
contadienoic acid; and alcohols: 2,2-dimethyl-3-pentanol, furfuryl alcohol, 2,3-butanediol,
4-hydroxybenzenemethanol, 2-pentadecanol, 1-heptadecanol, oleyl alcohol, 1-hexacosanol,
1-octacosanol and 1-dotriacontanol.

In the derivatization for the detection of aldehydes and alkynes, a total of 12 aldehydes
and three alkynes were identified. These corresponded to an increase in the total percentage
of identified compounds of 0.01% and 0.56% for EOT1 and EOT2, respectively. In summary,
the total percentages of identified compounds for EOT1 and EOT2 were 97.46% and 99.92%.
The compounds exclusively found in EOT2 were eight of the 12 aldehydes and the three
alynes (Table 5).

Table 5. Identified compounds of U. dermestoides essential oils derivatized by acetal and enol-
ether reaction.

| Compounds | EOUd1 RA (%) | KI Exp | EOUd2 RA (%) | KI Exp | KI Ref |
|-----------|--------------|--------|--------------|--------|--------|
| Aldehydes |              |        |              |        |        |
| 2 Hexanal | 0.001 ± 0.001 | 971.8  | 0.013 ± 0.001 | 968.5  | 964    |
| 3 Heptanal| 0.003 ± 0.000 | 1077.2 | 0.015 ± 0.001 | 1077.2 | 1069   |
| 4 Benzaldehyde | 0.021 ± 0.001 | 1107.0 | 0.015 ± 0.001 | 1107.0 | 1200   |
| 5 Phenylacetaldehyde | 0.161 ± 0.001 | 1217.2 | 1217.2 | 1194   |
| 6 Nonanal  | 0.001±0.001 | 1278.5 | 0.041 ± 0.001 | 1278.9 | 1267   |
| 7 Decanal  | 0.014 ± 0.002 | 1377.4 | 0.014 ± 0.002 | 1377.4 | 1366   |
| 8 Dodecanal| 0.013 ± 0.000 | 1477.4 | 0.013 ± 0.000 | 1477.4 | 1366   |
| 9 Tetradecanal | 0.001 ± 0.001 | 1744.4 | 0.051 ± 0.002 | 1744.4 | 1744.4 |
| 10 Pentadecanal | 0.012 ± 0.001 | 1849.8 | 0.012 ± 0.001 | 1849.8 | 1849.8 |
| 11 Hexadecanal | 0.068 ± 0.004 | 2017   | 0.068 ± 0.004 | 2017   | 2017   |
| 12 Octadecanal | 0.065 ± 0.010 | 2117.4 | 0.065 ± 0.010 | 2117.4 | 2117.4 |

In general, the compounds obtained from both oils can be classified into 10 categories:
alcohols, aldehydes, alkanes, alkenes, alkynes, alkyl disulfides, aromatic compounds,
carboxylic acids, and their derivatives, quinones and terpenes. The amount and type of
these metabolites varied depending on the stimulus to which the sample was subjected
when the essential oil was obtained. Despite a significant decrease in alkenes and terpenes
with respect to the peak area, the variety of these metabolites in EOT2 increased. For the
remaining metabolite groups, all compounds increased both in the peak area and in the
variety of compounds present (Figure 1).

One of the categories of greatest biological interest is terpenes; therefore, they were
analyzed independently. In EOT2, the number of functionalized terpenes increased, and
there was a tendency for the peak area to increase with respect to EOT1. Although in
terpenes that were not functionalized, the areas of the peaks were smaller, the variety of
terpenes present was greater in EOT2 than in EOT1 (Figure 2).
Figure 1. Grouped essential oil compounds. The data are presented as the median of the peak area of each compound (grouped by type) and the range of the data. * Significant difference (p ≤ 0.05).

Figure 2. Analysis of the amount and type of terpenes. (a) The data are presented as the median of the peak area of each terpene (grouped by type) and the range of the data. M: monoterpene, Mo: monoterpenoids, S: sesquiterpene, D: diterpene, Do: diterpenoids, St: sesterterpenes, Sto: sesterterpenoids, T: triterpene, To: Triterpenoids. * Significant difference (p ≤ 0.05). (b) Number of terpene compounds in each essential oil.

3. Discussion

Our results show that the four experimental conditions for the volatilome present three main compound groups: quinones, alkenes, and terpenes. The most abundant compounds are methyl-1,4-benzoquinone, ethyl-1,4-benzoquinone, limonene, 1-pentadecene, and 1-tridecene, in agreement with previous reports [6,7]. However, we found 15 terpenes, four quinones, two alkenes, and four aromatic compounds that had not been previously identified in this organism. The HS-SPME results show the presence of a complex mixture of metabolites of different chemical nature, and the changes over time may be a reflection...
of the metabolic variety and/or an effect of the equilibrium absorption-desorption process of the compounds in the fiber.

For essential oils, there are five groups of major compounds, these being alkenes (1-pentadecene), carboxylic acids (palmitic, myristic, oleic, and linoleic acids), alkanes (pentacosane and hentriacontane), terpenes (limonene, dehydroabietic acid, β-sitosterol), and alcohols (2-heptanol). 1-Pentadecene is the main component in both essential oils, contrary to the previous report in HS-SPME, where EBQ and MBQ are reported as the main components [6]. This alkene is reported for some coleopters, and it is hypothesized as an epideictic pheromone and defensive secretion [16]. However, other compounds were identified in both analyses, including 50 other terpenes, 37 carboxylic acids, and their derivates, 16 alkanes, nine alkenes, three alkynes, 18 alcohols, 12 aldehydes, nine alkyl disulfides, four quinones, and six aromatic compounds. Note that some terpenes, hydroquinones, and carboxylic acids have been previously reported for other coleopters [17–19] but never before, until the current work, for *U. dermestoides*.

Regarding the relative percentage of the identified VOCs, the results obtained in this study suggested a considerably lower rate of release of quinone derivatives by *U. dermestoides* in the presence of the PBET solution than in its absence, and this trend was independent of the type of fiber used for the analysis. However, when analyzing the concentrations of these metabolites in the essential oil, it was observed that their concentration was considerably lower than expected with respect to HS-SPME. This finding indicates that HS-SPME results tend to depend on the balance in the absorption-desorption process, which does not guarantee that the metabolites best captured by the fibers are the most abundant in the sample. In addition, as in other studies [20–22], the components identified by hydrodistillation are greater than those obtained by HS-SPME. Therefore, despite the shorter analysis time and the preservation of the sample, HS-SPME remains a limited tool for the characterization of a large number of the compounds present in a complex sample.

However, the fact that quinones are present at low concentrations in essential oils is encouraging, since the importance of these metabolites lies in their potential toxicity. The toxicity of quinones to cells is based on a series of mechanisms that include oxidative stress, redox cycles, arylation, intercalation, induction of cuts in DNA chains, generation of free radicals, and interference with mitochondrial respiration [23–25].

In insects, terpenes play essential roles as sex pheromones, trail pheromones, and aggregation and alarm pheromones, as well as in the defense against pathogens [26,27]. It has been postulated that insects can synthesize them de novo, generally as monoterpenes, and they also have the ability to sequester terpenes produced by host plants or endosymbiotic microorganisms [26–28]. Monoterpenes are presumably assembled from isopentenyl diphosphate (IDP) and dimethylallyl diphosphate (DMADP) derived from the mevalonate route. In this metabolic pathway, the *trans*- or *cis*-isoprenyl diphosphate synthases (IDSs) catalyze the condensation of IDP with one or two isomers of DMADP [26–30]. *Trans*-IDS enzymes have the particularity of being able to catalyze the syntheses of both precursors and final metabolites, and they can also produce monoterpenes and sesquiterpenes, depending on the cofactor to which they are exposed [29–31]. This protein is expressed to a greater extent in insect fat bodies, so this tissue may be the location of the syntheses of terpenes or their precursors [28,32]. Wherever terpenes and/or their precursors are synthesized, they are transported by the hemolymph to reservoir glands, where they are released as part of a defense mechanism [28,29].

Our results suggest that the increase in the size of terpenes produced by *U. dermestoides* could be explained by the release of cofactors that regulate the activity of IDSs via stimulation of simulated gastric juice. The acidic environment produced by this stimulus could improve the bioavailability of metal ions to the beetle and thereby modify the ability of these proteins to regulate activity. Likewise, it has been reported that IDS proteins can remain active over wide ranges of pH (pH 4–8) and temperature (15–45 °C) [29], so they could be active even after the incubation of the beetle with the PBET solution. Although this could tell us how the insect produces terpenes of greater molecular weight, there are no
reports in the literature detailing the mechanisms by which an insect modifies the functionalization of the terpenes it produces. This process has been well documented in plants [33]; however, many of the processes and enzymes involved in the metabolomics of insects are still unknown. Likewise, the fact that terpenic acids—which are produced by conifers as well as some species from Asteraceae, Celstraceae, Hydrocharitaceae, and Lamiaceae, even some cyanobacterial and fungal species [34,35]—have been detected lays the foundation for rethinking whether the insect not only assimilates these metabolites from food [36,37] but would also be capable of producing them.

As with terpenes, alkanes and alkenes in insects are produced in specialized cells called oenocytes, which are found mainly in the abdomen—associated with epidermal cells or, in some cases, with body fat cells [38,39]. Hydrocarbons are subsequently transported by the hemolymph to both external and internal tissues, including the epicuticle, fat body, ovaries, and reservoir glands [39–41]. Cuticle hydrocarbons in insects have two main functions: to protect the insect against desiccation and as signaling molecules in a wide variety of chemical communication systems [42,43].

In our results, a considerable increase in long-chain fatty acids (myristic acid, palmitic acid, stearic acid), saturated aldehydes, and methyl-branched and saturated alcohols were observed in EOT2 compared to EOT1. This could be explained by a modification of the metabolic pathways of cuticle hydrocarbon production by the stress conditions to which the insects were subjected in EOT2 to attempt to protect the insect from the hostile environment to which it was subjected. Therefore, we observe how these precursors (long-chain fatty acids, aldehydes, and alcohols)—as well as the final product of the metabolic route n-alkanes and methyl-branched alkanes—increase. Considering that alkenes have a lower melting point as well as a lower impermeability profile that could affect survival [44], the insect modifies its metabolic routes to enhance the production of alkanes. The absence of the precursors of n-alkenes, unsaturated alcohols, and aldehydes would explain why EOT2 does not increase the number of alkenes identified in the essential oil.

As with the previous metabolites, the increase in the concentration of alkyl disulfides may be a response to the exposure of insects to PBET. However, it is not yet clear how these metabolites are produced or what function they have in the insect.

Many of the metabolites found in *U. dermestoides* have a prior history of clinically important biological activity, among which we can highlight azelaic acid, furfuryl alcohol, benzaldehyde, and phenylacetaldehyde. These compounds possess numerous biological activities of clinical interest, such as anti-inflammatory, antimicrobial, antioxidant, antifungal, and anticancer properties [45–48]. However, the group of compounds with the greatest diversity of biological activities of interest are terpenes such as limonene, fucosterol, and dehydroabietic acid. These terpenes present antioxidant, anticancer, antiulcer, antihistaminic, antiadipogenic, antiphotodamaging, antimicrobial, antitumor, gastroprotective, hepatoprotective, antiviral, antihyperalgesic, anti-inflammatory, anticholinergic, anti-osteoporotic, anti-diabetic, and anti-hyperlipidemic activities [49–51].

4. Materials and Methods
4.1. Chemicals

The reagents used in this study were sodium citrate, lactic acid, pepsin, N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), trimethylsilyl chloride (TMCS), boron trifluoride methanol solution (Sigma-Aldrich, St Louis, MO, USA), DL-malic acid, acetic acid and ethylc ether (JT Baker, Deventer, Holland).

4.2. Insects

*Ulomoides dermestoides* were originally obtained from a local provider. The taxonomical identity of *U. dermestoides* was obtained according to the keys published by Kim and Jung (2005) [52], in the Insecticidal Natural Compounds Laboratory of the Faculty of Chemistry, Autonomous University of Querétaro, México. A two-year-old colony was maintained at
27 ± 2 °C and 70 ± 5% relative humidity on a sterile oatmeal substrate and fed with whole bread supplemented with banana peels.

4.3. Sample Preparation

Five adult insects were gently placed in a 17 mL glass vial sealed with a Teflon cover with a rubber septum. The samples were incubated and evaluated at five different time points (5 min, 1 h, 6 h, 18 h, and 24 h) in order to monitor changes in the profile of the volatilome of the insect. In addition, the insects were subjected to two treatments, and each condition was tested in duplicate:

- Treatment 1 (T1): manually shaking the vial for 5 min at room temperature to stimulate the release of defense secretion.
- Treatment 2 (T2): 1.5 mL of the PBET solution was added to the vial with the insects and incubated at 37 °C with constant agitation (130 rpm). The PBET solution consisted of 0.5 mg/mL sodium citrate, 0.5 mg/mL malic acid, 0.5 µL/mL acetic acid, 0.4 µL/mL lactic acid and ~800 U/mL pepsin at pH 3. The PBET solution simulates the leaching of a solid matrix in the human gastrointestinal tract in order to determine the bioaccessibility of a particular element, such as the total fraction available for adsorption during transit through the small intestine [53]. This digestion simulant solution allowed emulation of the conditions of the insects being ingested and digested by gastric fluid.

4.4. VOCs Collection by HS-SPME

The HS-SPME technique was performed using a 75-µm film thickness carboxen/polydimethylsiloxane (CAR/PDMS) and 60-µm film thickness carbowax (PEG) fibers (Supelco, Bellefonte, PA, USA) to detect compounds from nonpolar to polar. To sample the VOCs secreted by *U. dermestoides*, the fibers were placed at a constant distance of 3.4 cm from the insects in treatment 1 and 2.6 cm from the insects in treatment 2. VOCs were absorbed for 15 min, and a desorption time of the fibers of 15 s was used. Fibers were previously conditioned for 5 min at 250 °C at the injection port and reconditioned before each analysis.

4.5. Volatilome GS-MS Analysis

GC-MS analysis was performed using a 6890N Network GC System coupled to a 5973 Network mass selective detector (MSD) (Agilent Technologies, Wilmington, DE, USA). The separation was performed using an HP-5MS capillary column (0.25 mm i.d. × 30 m, 0.25 µm film thickness) (J&W, Folsom, CA, USA). The injector was operated in the splitless mode at 250 °C, and the oven temperature was programmed to be 40 °C for 3 min, and then heated at 15 °C/min to 250 °C with a holding time of 5 min at the final temperature. The MSD was operated at 70 eV, the ion source was set at 150 °C, and the transfer line was at 250 °C. VOCs were identified by interpreting their mass spectra fragmentation in the mass range of 50–400 atomic mass units. The software MSD ChemStation (Agilent B.04.02) was used for data recording. The compounds were identified by comparing the obtained mass spectra with those of reference compounds from the National Institute of Standards and Technology (NIST11) and Wiley 9th. The identities of the compounds were confirmed by the Kovats retention index calculated for each peak with reference to the *n*-alkane standards (C7–C18) running under the same conditions.

4.6. Obtention of Essential oil of *U. dermestoides* (EOT1)

An amount of 306 g of adult *U. dermestoides* was hydrodistilled at the boiling temperature of the water. The VOCs were extracted from the stripping water by means of liquid-liquid extraction with ethyl ether. The organic phase was concentrated at 20 °C under reduced pressure until the solvent was eliminated, and the residual water was removed with sodium sulfate.
4.7. Obtention of Essential oil of U. dermestoides Post PBET Digestion (EOT2)

An amount of 383 g of adult U. dermestoides was digested for 12 h in PBET solution with subsequent inactivation of the solution to pH 7 with sodium bicarbonate. The VOCs were extracted from the stripping water by means of liquid-liquid extraction with ethyl ether. The organic phase was concentrated at 20 °C under reduced pressure until the solvent was eliminated and the residual water was removed with sodium sulfate.

4.8. Derivatization for Alcohols and Carboxylic Acid Detection

Essential oils were diluted to 2% in 500 µL heptane and introduced into a 10 mL vial. Then, 100 µL of BSTFA/TMCS solution (9:1 v/v) was added to the same vial as a silanizing agent. The mixture was reacted at 80 °C under microwave irradiation (200 W microwave power) for 10 min using the Discover System 908,005 (CEM Corporation, NC, USA).

4.9. Derivatization for Aldehydes and Alkyne Detection

Essential oils were diluted to 2% in 500 µL heptane and introduced into a 10 mL vial. Then, 100 µL of boron trifluoride 14% in methanol solution was added to the same vial. The mixture was reacted at 80 °C under microwave irradiation (200 W microwave power) for 10 min using the Discover System 908005.

4.10. Essential Oil GS-MS Analysis

Samples without derivatization were diluted to 2% in heptanol, using 1 µL of each sample for the analysis, and each sample was analyzed in triplicate. GC-MS analysis was performed using a 7890A Network GC System coupled to a 5975C Network mass selective detector (MSD) and 7683B autosampler (Agilent Technologies, Wilmington, DE, USA). The separation was performed using an HP-5MS capillary column (0.25 mm i.d. × 30 m, 0.25 µm film thickness) (J&W, Folsom, CA, USA). The injector was operated in splitless mode at 300 °C, with a flow of 0.8 mL/min, and the oven temperature was programmed to 40 °C for 3 min, and then heated at 3 °C/min to 300 °C with a holding time of 5 min at the final temperature. The MSD was operated at 70 eV; the ion source was set at 150 °C and the transfer line at 300 °C. VOCs were identified by interpreting their mass spectra fragmentation in the mass range of 15 to 800 atomic mass units. The software MSD ChemStation (Agilent) was used for data recording. The compounds were identified by comparing the obtained mass spectra with those of reference compounds from the National Institute of Standards and Technology (NIST11) and Wiley 9th. The identities of the compounds were confirmed by the Kovats retention index calculated for each peak with reference to the n-alkane standards (C7–C38) running under the same conditions.

4.11. Statistical Analysis

The relative percentage of each metabolite was calculated considering the peak area obtained by GC-MS of each metabolite in relation to the total area of peaks analyzed. Data represent the mean of the relative percentage of three repeats ± SD. Metabolites grouped for type for each essential oil were compared with the Mann Whitney U test considering the peak area of each metabolite and a p ≤ 0.05. The data in the graphics were expressed as median and range of each group. GraphPad Prism 5 was used to perform the analysis.

5. Conclusions

In the volatilome analysis, the use of fibers of different polarities was necessary to expand the detection of metabolites in U. dermestoides. Under these analytical conditions, we found 15 terpenes, four quinones, two alkenes, and four aromatic compounds that had not been previously identified in this organism. The composition of essential oils consisted of 10 groups of compounds: alcohols, aldehydes, alkanes, alkenes, alkynes, alkyl disulfides, aromatic compounds, carboxylic acids, and their derivatives, quinones, and terpenes. There were 146 metabolites not previously reported for U. dermestoides, in addition to those identified by HS-SPME, of which 76 were found in EOT1 and 132 in EOT2. Between both
studies approaches a total of 203 compounds were identified, of which 171 metabolites are reported for the first time in this work for *U. dermestoides*.

In addition, the exposure of *U. dermestoides* to PBET solution in both study approaches showed modifications in the expression of secondary metabolites, principally, an increase in the number of alkanes, alkynes, aromatic compounds, alcohols, alkyl disulfides, carboxylic acids, and terpenoids.

This work reports essential oils obtained from insects for the first time, and also, lays the foundations for the bio-directed study of entopharmacological activity and metabolic pathways of *U. dermestoides* essential oils and their metabolites.

**Supplementary Materials:** The following are available online. Figures S1–S6 show representative chromatograms of the volatilome and essential oils of *U. dermestoides*.

**Author Contributions:** Conceptualization, C.G.C. and M.M.G.-C.; methodology, P.J.C.-S., C.G.C., M.A.R.-L. and M.M.G.-C.; formal analysis, P.J.C.-S. and M.M.G.-C.; investigation, P.J.C.-S., C.G.C. and M.M.G.-C.; writing—original draft preparation, P.J.C.-S.; writing—review and editing, P.J.-S., C.G.C., M.A.R.-L. and M.M.G.-C. All authors have read and agreed to the published version of the manuscript.

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