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**Characterization, antioxidant activity, and inhibitory effect on pancreatic lipase of extracts
from the edible insects *Acheta domesticus* and *Tenebrio molitor***

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19 **ABSTRACT**

20 Extracts from the edible insects *Acheta domesticus* and *Tenebrio molitor* were obtained by
21 ultrasound-assisted extraction (UAE) and pressurized-liquid extraction (PLE) using ethanol (E) or
22 ethanol:water (E:W). Characterization by GC-MS was performed and total phenolic compounds
23 (TPC), antioxidant activity (DPPH) and pancreatic lipase inhibitory capacity were assayed.
24 Most extracts, mainly ethanolic extracts, predominantly presented lipids as free fatty acids,
25 followed by aminoacids, organic acids, carbohydrates, hydrocarbons and sterols. The UAE-E:W
26 extracts were different, being characterized by organic acids for *A. domesticus*, or aminoacids for *T.*
27 *molitor*. All the extracts exhibited antioxidant activity, which correlated with TPC values, being the
28 E:W extracts the most effective. All the extracts showed inhibitory activity of lipase, although those
29 from *T. molitor* and extracted by PLE were the most effective.
30 Therefore, bioactive insect extracts can be selectively obtained by advanced methods of extraction,
31 being aqueous ethanol preferred for antioxidant activity and PLE for inhibitory lipase activity.

32
33 **Keywords:** edible insects, *Tenebrio molitor*, *Acheta dometicus*, ultrasound-assisted extraction,
34 pressurized-liquid extraction, bioactive compounds, antioxidant, pancreatic lipase

35

36 1. INTRODUCTION

37 Edible insects have gained a remarkable attention in Western countries over the last years,
38 especially after FAO's recommendations to include certain species of insects in Western diets as a
39 novel alternative to traditional animal sources of proteins and fats in order to cover the nutritional
40 requirements of the population without causing a great environmental impact (van Huis & Oonincx,
41 2017). In fact, the European Food Safety Authority (EFSA) included whole insects and their parts
42 as novel foods in 2018, enabling the safe and regulated introduction of edible insects in the
43 European market. A list of insect species with a strong potential to be used as food and feed in the
44 European Union has been recently proposed, including: *Tenebrio molitor*, *Acheta domesticus*,
45 *Musca domestica*, *Gryllodes sigillatus*, *Alphitobius diaperinus*, *Hermetia illucens*, *Zophobas*
46 *atratus*, *Achroia grisella*, *Bombyx mori*, *Locusta migratoria migratorioides*, *Galleria mellonella*
47 *and Schistocerca americana* (EFSA Scientific Committee, 2015).

48 Most of the commercial insect presentations are being developed as whole insects or insect flours
49 for their incorporation in food products, mainly claimed as protein sources. Nevertheless, the
50 exploration of other alternative forms of insect presentations for human consumption, which are
51 rich in other diverse compounds different to proteins, has been scarcely considered. In this sense,
52 the production of specific insect extracts, might lead to concentrated forms of insects, rich in
53 diverse compounds of potential interest different to proteins, such as fibers, lipids or minor
54 compounds. In this last case, minor compounds might include bioactive molecules of potential
55 functional interest, which might be worthy to study to potentiate the insect-based food products.

56 Among edible insects, ~~despite~~ *although* the available information is still scarce, a diversity of
57 bioactivities linked to certain bioactive compounds are being intensively described in the literature
58 in the last years, such as antiinflammatory, antimicrobial, antiangiogenic, antiproliferative or
59 antioxidant, although the specific mechanism or compounds responsible for such bioactivity have
60 not been clearly elucidated in most cases (Seabrooks & Hu, 2017). As an example, Zielińska, Karaś,
61 & Jakubczyk (2017) described an antioxidant and antiinflammatory effect of peptides obtained from

62 *T. molitor*, *Schistocerca gregaria* and *Gryllodes sigillatus*. The antiinflammatory activity of other
63 insects such as *Lycorma delicatula* or *Holotrichia diomphalia* has also been evidenced (Baek et
64 al., 2018; Hong, Kim, & Lee, 2019). Phenolic compounds seem to play an important role in the
65 antioxidant effect of insect extracts, as demonstrated by Liu et al. (2012) for an ethanolic extract of
66 *Holotrichia parallela*. Other authors have also described the antioxidant effect of different insect
67 species (Hong et al., 2019; Hwang et al., 2019; Li et al., 2017; Tang et al., 2018), but such activity
68 was not ascribed to specific compounds. *In vivo* studies in mice fed with diets rich in fat have also
69 shown an antiadipogenic and antiobesity effect of aqueous solutions obtained from *T. molitor* and
70 *Allomyrina dichotoma* (Seo et al., 2017; Yoon et al., 2015). Additionally, Ali & Arumugam (2011)
71 described a positive effect on hypercholesterolemia and atherosclerosis in rabbits fed with extracts
72 of *Bombyx mori* cocoons. Concerning the effects related to the hypolipidemic activity of insects, the
73 specific mechanism has not been elucidated. However, it is known that one of the mechanisms of
74 natural bioactive compounds against overweight, hypertriglyceridemia or hypercholesterolemia is
75 the inhibition of the digestive enzyme of dietary lipids, the pancreatic lipase (Herrera, Navarro del
76 Hierro, Fornari, Reglero, & Martin, 2019a). However, to the best of our knowledge, the ability to
77 inhibit the pancreatic lipase enzyme has not been described for edible insects or concentrated forms
78 presented as insect extracts, which might of great interest.

79 Concerning the exploration of insect extracts, conventional extraction techniques, such as Soxhlet
80 and maceration, have been used as procedures to deepen only into the nutritional composition of
81 insects and fatty acid profile (Musundire, Zvidzai, Chidewe, Samende, & Manditsera, 2014;
82 Tzompa-Sosa, Yi, van Valenberg, van Boekel, & Lakemond, 2014; Yi et al., 2013). These
83 conventional techniques, however, are time-consuming; require high purity solvents and display
84 low extraction selectivity and efficiency (Nguyen, Pham, Bowyer, Altena, & Scarlett, 2016).
85 Therefore, non-conventional extraction technologies, which require reduced extraction times,
86 energy consumption and have a higher extraction efficiency, are being widely used as greener
87 techniques to obtain natural extracts, generally from plant materials (flowers, seeds, leaves or roots)

88 (Azmir et al., 2013). Among these non-conventional techniques, the ultrasound-assisted extraction
89 (UAE) and pressurized liquid extraction (PLE) have gained attention as solid-liquid extraction
90 processes due to the abovementioned advantages. In UAE, the cavitation phenomenon of bubbles
91 caused by sonication enables a higher transfer of mass to the solvent, whilst in PLE, the high
92 temperatures and pressures enable the fast and efficient extraction of compounds thanks to a
93 reduction in the polarity, viscosity and surface tension (Conte et al., 2016; Da Porto, Porretto, &
94 Decorti, 2013). Very few works have been done regarding the obtention of extracts from edible
95 insects by non-conventional techniques. The ultrasonic-assisted aqueous extraction has been
96 performed recently by Sun et al. (2018) to obtain a functional oil from the edible larvae of *Clanis*
97 *bilineata*. Liu et al. (2012) performed microwave-assisted extraction of phenolic compounds and
98 proteins from the edible beetle *Holotrichia parallela* Motschulsky by using ethanol and water as
99 solvents. As far to our knowledge, the PLE extraction has not been previously tested for insect
100 matrices.

101 The aim of this work was to obtain extracts from two edible insects, *Tenebrio molitor* and *Acheta*
102 *domesticus*, by ultrasound-assisted extraction (UAE) and pressurized liquid extraction (PLE) using
103 ethanol or aqueous ethanol as solvents, and to further compare the composition of the obtained
104 extracts by gas chromatography-mass spectrometry. Subsequently, the *in vitro* antioxidant activity
105 and inhibitory capacity of the extracts against pancreatic lipase were evaluated.

106

107 2. MATERIALS AND METHODS

108 2.1 Raw materials and chemicals

109 *A. domesticus* (adult) and *T. molitor* (larvae) were purchased frozen in a local company specialized
110 in insect production intended for animal feed (Animal Center SL, Valencia, Spain).

111 Absolute ethanol (131086.1214), sodium carbonate (131648.1210) and Folin-Ciocalciu reagent
112 (A5084,0500) were purchased from Panreac (Barcelona, Spain). Methanol (6712-25) and
113 dimethylsulfoxide (DMSO) (LC1334) were purchased from Macron (Poland) and Lab-Scan

114 (Dublin, Ireland), respectively. N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) (15238), gallic
115 acid (G7384), 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]) (257621), Dulbecco's Phosphate Buffered
116 Saline (PBS) (59300C), lipase from porcine pancreas (L3126) and 4-methylumbelliferyl oleate (4-
117 MUO) (75164) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

118

119 **2.2 Obtention of the extracts**

120 Prior to extraction, insects were gently rinsed with distilled water, freeze-dried (LyoBeta 15,
121 Telstar, Terrasa, Spain), ground in a knife mill (particle size < 500 µm) (Grindomix GM 200,
122 Retsch GmbH, Haan, Germany), kept in sealed bags and stored at -20 °C protected from oxygen,
123 light and moisture until further use. The lyophilized insect flours were submitted to two extraction
124 methods: ultrasonic assisted extraction (UAE) and pressurized liquids (PLE) with two solvents of
125 different polarity: ethanol (E) and ethanol:water (1:1, v/v) (E:W) in a sample/solvent ratio of 1:10
126 (w/v). All extractions were performed in duplicate. Extracts were stored at -20 °C until further use.

127

128 **2.2.1. Ultrasound-assisted extraction (UAE)**

129 Extractions were carried out by direct sonication (Branson SFX250 Digital Sonifier, Branson
130 Ultrasonics, USA) with an ultrasonic probe (1/2" diameter) for 15 min at a sonication output
131 amplitude of 60% in continuous pulse by direct sonication at 20 kHz, as described by Navarro del
132 Hierro et al. (2018). The temperature during the extraction process was kept under 70 °C. The
133 mixture was then centrifuged at 4500 rpm for 10 min. The ethanol contained in the supernatant was
134 dried under vacuum using a rotary evaporator, whilst the aqueous fraction was lyophilized for the
135 E:W extracts.

136

137 **2.2.2. Pressurized liquid extraction (PLE)**

138 Extractions were performed using an accelerated solvent extractor (ASE, 350, Dionex Corp,
139 Sunnyvale, CA, USA) equipped with a solvent controlled unit. 2 g of insect flour were loaded into

140 the stainless-steel cell with sea sand (thin grain, particle size 250 – 300 µm, Sigma-Aldrich, Madrid,
141 Spain) above and below the sample to avoid any void spaces. Extractions were performed at 120 °C
142 for 15 min and 100 bars and using N₂ as a compressor gas. The ethanol contained in the samples
143 was dried under vacuum using a rotary evaporator, whilst the aqueous fraction was lyophilized for
144 the E:W extracts.

145

146 2.3. Analysis of the extracts by GC-MS

147 The ~~composition of the~~ extracts were characterized by GC-MS after derivatization of the samples
148 with BSTFA according to Herrera et al. (2019b) with small modifications. Extracts were dissolved
149 in BSTFA at a concentration of 20 mg/mL and heated at 75°C for 1 h. After, samples rested at room
150 temperature for 5 minutes and then were centrifuged for 5 minutes at 4500 rpm (Centrifuge
151 MiniSpin® plus). The supernatant was analyzed in an Agilent 7890A GC-MS (Agilent
152 Technologies, Santa Clara, CA, USA). The column employed was an Agilent HP-5MS UI capillary
153 column (30 m × 0.250 mm × 0.25 µm) and the carrier gas was helium with a flow of 2 mL/minute.
154 A G4513A autoinjector was used, with 1 µL injections in splitless mode and the injector
155 temperature was 260°C. The oven was initially set at 50 °C and increased at 10 °C/minute to 310 °C,
156 held for 25 minutes. The inlet temperatures at the MS were set at 260 °C and those at the MS ion
157 source and the interface were 230 °C and 280 °C, respectively. The scanning speed was 0.79 scans/s
158 in a mass range of 30-1000 amu. Identification of compounds was performed by the NIST MS Data
159 library, by the mass spectra according to literature, or according to commercial standards (most
160 fatty acids, glycerides, sugars, and sterols), previously derivatized following the same procedure as
161 samples.

162

163 2.4. Total Phenolics Content (TPC)

164 The content of total phenolic compounds (TPC) of the extracts was determined by the Folin-
165 Ciocalteu colorimetric test developed by Singleton, Orthofer, & Lamuela-Raventós (1999) with

166 some modifications. 10 µL of extracts at 10 mg/mL in DMSO were mixed with 600 µL of Milli-Q
167 water and 50 µL of Folin reagent, and allowed to stand for 1 minute. Next, 150 µL of a 20% (w/v)
168 sodium carbonate solution and 190 µL of Milli-Q water were added. After incubation in darkness
169 for 1 h, the absorbance was measured at 760 nm. The results were expressed as g of gallic acid
170 equivalents (GAE) per 100 g of extract using a standard curve of gallic acid (ranging from 12.5 to
171 1000 µg/mL). Determinations were done in triplicate.

172

173 **2.5. Antioxidant activity of the extracts by DPPH assay**

174 The antioxidant activity of the extracts was measured by the DPPH[·] assay, described by Blois
175 (1958). The extracts were dissolved in DMSO at 10 mg/mL. 40 µL were mixed with 560 µL of a
176 solution of DPPH[·] in methanol (0.06 mM). The samples were homogenized and incubated at room
177 temperature for 60 minutes in darkness. The absorbance was measured at 517 nm and the control
178 used was DMSO. Control samples were prepared in the absence of extracts, following the same
179 procedure. The remaining DPPH[·] concentration of all samples was calculated from a DPPH[·]
180 calibration curve. The determinations were made in duplicate. The antioxidant activity was
181 expressed as a percentage of DPPH[·] inhibited by the following formula:

$$182 \quad \% \text{ Inhibition DPPH}^{\cdot} = 100 - \left(\frac{\mu\text{g DPPH}^{\cdot} / \text{mL}_{\text{sample}}}{\mu\text{g DPPH}^{\cdot} / \text{mL}_{\text{control}}} \times 100 \right)$$

183

184 **2.6. Pancreatic lipase inhibition assay**

185 The inhibitory activity of each extract against pancreatic lipase was measured by using 4-MUO as
186 substrate, according to Herrera et al. (2019a) with modifications. E:W extracts were previously
187 diluted in PBS and the E extracts were diluted in a PBS/DMSO solution (1.7:1 v/v). The reaction
188 mixture consisted of 500 µL of extract solution at different concentrations, 500 µL of freshly-
189 prepared pancreatic lipase at 1 mg/mL (0.01 g of lipase in 10 mL PBS, stirred for 10 min and
190 centrifuged at 4000 rpm for 10 min), and lastly, 1 mL of 4-MUO solution at 0.1 mM in PBS.
191 Control samples in absence of extracts were prepared following the same procedure. Triplicates

were made for each of the samples and for each of the extracts concentrations.

The reaction mixture was placed in an orbital incubator at 250 rpm and 37 °C, for 20 minutes. After incubation, three aliquots of 150 µL were taken and added to a 96-well plate. The amount of 4-MUO hydrolyzed by lipase was measured in a 96-well microplate using a fluorescence microplate reader (Polarstar Galaxy, BMG Labtechnologies) at an excitation wavelength of 350 ± 10 nm and an emission wavelength of 450 nm. The inhibition of pancreatic lipase activity was calculated as follows:

$$\% \text{ Inhibition Lipase} = 100 - \left(\frac{\text{Fluorescence}_{\text{sample}}}{\text{Fluorescence}_{\text{control}}} \times 100 \right)$$

Finally, a logarithmic regression curve was established to calculate IC₅₀ values (mg/mL), defined as the concentration of the extract that inhibited 50% the activity of the pancreatic lipase.

2.7. Statistical analysis

Statistical analyses were performed by means of the general linear model procedure of the SPSS 24.0 statistical package (SPSS Inc., Chicago, IL, USA) by one-way analysis of variance. Differences were considered significant at $p \leq 0.05$. Post-hoc Tukey's tests were performed in order to establish significant differences.

3. RESULTS AND DISCUSSION

3.1. ~~Characterization~~ GC-MS analysis of the edible insect extracts

~~A preliminary and general characterization~~ The GC-MS analysis of the PLE and UAE extracts from both insect species and with both solvent extractions was performed by GC-MS following previous formation of trimethylsilyl derivatives of all those less volatile compounds containing hydroxyl or carboxyl functional groups. This procedure allowed to tentatively identify ~~up to~~ 89 compounds for *A. domesticus* and 97 compounds for *T. molitor* (Table 1 and 2, respectively). Compounds were categorized ~~in~~ into 12 groups depending on their principal chemical family. Thus, lipids, nitrogen compounds, organic acids, carbohydrates, sterols and hydrocarbons were identified. In order to

enhance the general comparison of the extracts, the total chromatographic area of the major chemical groups detected is shown in Figure 1.

~~In general~~ For most of the extracts, and regardless of the insect species, the highest percentage of area corresponded to lipids, and specially, to free fatty acids (Tables 1 and 2). Within these fatty acids, the highest percentage corresponded to 9,12-octadecadienoic acids, followed by hexadecanoic acid. These are typical fatty acids frequently described for *A. domesticus* and *T. molitor* within their lipid fraction (Paul et al., 2017; Tzompa-Sosa et al., 2014). Glycerides under the form of monoglycerides and diglycerides were also detected in both species. The obtained results might be expected, since these insect species are known for their high fat content and the extraction conditions might favor the concentration of a major lipid fraction. Nevertheless, some specific differences were observed between the insect species, and regardless of the conditions of extraction. The *A. domesticus* extracts tended to contain higher proportions of organic acids, non-protein nitrogen compounds, sugars, and other minor compounds, such as sterols, compared with *T. molitor*. On the contrary, the *T. molitor* extracts tended to contain higher proportions of fatty acids, glycerides and aminoacids, as well as other minor compounds such as cholecalciferol and alkanes. Additionally, the diversity of compounds was higher for *T. molitor*, especially in the number of different fatty acids and aminoacids detected. Therefore, although most of the same chemical compounds were detected in both species, the general quantitative and qualitative composition of the extracts from both species was slightly different.

Regardless of these general differences between species, the different conditions of extraction also caused variations in the chromatographic profile of the extracts. Concerning the major detected fraction of lipids, ~~this~~ the variation was especially high for all the ethanolic extracts obtained by both UAE and PLE, accounting for more than 80% of the total chromatographic areas (Tables 1 and 2 and Figure 1). This result might be expected, since the used conditions of extractions might favor the extraction of non-polar fractions, especially with the less polar solvents, such as ethanol. Therefore, ~~these obtained~~ results suggested that the assayed conditions of extraction might allow to

244 obtain concentrated forms of free fatty acids of insects, mainly under the form of linoleic acid.

245 It is interesting to remark that only in ~~the~~ case of the UAE-E:W extracts from both insects, ~~the~~ lipids

246 were not the major detected ~~family of~~ compounds, being minor in these extracts (Figure 1). Thus, in

247 ~~the~~ case of UAE-E:W extracts of *A. domesticus*, the major percentage of area corresponded to

248 organic acids (around 40% of total area); being gluconic acid and its derivatives the most abundant

249 (around 36% of total area) (Table 1). Gluconic acid is a mild organic acid derived from the

250 oxidation of glucose. ~~This~~ It is frequently found in plants, fruits, wine, vinegar or honey, as well as

251 ~~derived~~ ~~produced~~ from fermentation processes by microorganisms, catalyzed by glucose oxidase or

252 glucose deshydrogenase (Ramachandran, Fontanille, Pandey, & Larroche, 2006). Concerning

253 insects, the available information is scarce, but the natural presence of gluconic acid and derivatives

254 was described in the composition of the defensive secretion of some insects (Farine, Everaerts,

255 Abed, & Brossut, 2000). The relevance of this compound is that gluconic acid is authorized as ~~a~~

256 food additive (E-574) and is extensively used in foods due to its different technological properties,

257 as flavoring or leavening agent, ~~so there is being~~ a relevant industrial production of this acid ~~with~~

258 the market of organic acids for foods (Ramachandran et al., 2006). ~~Due to~~ ~~For~~ all these reasons, the

259 obtained results were of great interest ~~because, as far to our knowledge since,~~ as far as we know,

260 this is the first time that the production of gluconic acid-rich extracts from insects after ~~advanced~~

261 ~~extraction~~ procedures ~~of~~ has been described. However, further studies would be necessary in order

262 to understand whether the assayed insects would be a natural source of this organic acid, or whether

263 this was the result of an undesirable fermentation of the assayed samples. Additional quantification

264 would be also necessary in order to properly estimate the ~~concentration of this~~ organic acid ~~in~~ the

265 extracts.

266 Furthermore, other relevant compounds detected in the UAE-E:W extracts of *A. domesticus* were

267 aminoacids (around 25% of total area), sugars (around 14% of total area) and non-protein nitrogen

268 compounds (around 9% of total area). In the specific case of aminoacids, six essential aminoacids

269 were detected (accounting 8% of chromatographic area), arranged by decreasing order of area

percentage as follows: lysine > valine > leucine > histidine > threonine > phenylalanine.

The UAE-E:W extracts from *T. molitor* were also different compared with the rest of the extracts of this specie (Figure 1). The major percentage of area corresponded to aminoacids (around 54% of total area) (Table 2). Therefore, these extracts were twice richer in aminoacids than the same extracts of *A. domesticus*. The six essential aminoacids detected (19% of chromatographic area) were arranged in decreasing order of area percentage as follows: valine > histidine > lysine > tryptophan > phenylalanine > threonine. Therefore, regardless of the insect species, the aminoacid enrichment observed for both UAE-E:W extracts, ~~should be remarked, suggesting~~ suggests the potential to produce free aminoacid-rich extracts from insects ~~by~~ through advanced methods of extraction for food purposes.

Additionally, other relevant compounds detected in the UAE-E:W extracts of *T. molitor* were sugars (around 13% of total area), non-protein compounds (around 10% ~~of total area~~) and organic acids (around 8% ~~of total area~~).

As a summary, ~~despite-although an~~ exhaustive quantification might be necessary to clearly state the specific concentration of different compounds in the extracts, the results obtained for the UAE and PLE ethanolic extracts from both insects suggest that non-polar compounds, such as lipids, might be within the major components of these extracts. On the contrary, both insect extracts obtained by UAE-E:W might be mainly ~~characterized-composed~~ by more polar compounds, such as organic acids for *A. domesticus* extracts or aminoacids for *T. molitor* extracts. However, it is important to remark that the analytical procedure used to characterize these extracts just shows a partial characterization of small to medium compounds containing –OH or –COOH functional groups. Therefore, despite the great diversity of compounds detected and the interesting comparisons in which the extraction conditions and insect species were considered, an exhaustive characterization by other advanced analytical tools would be of further interest in order to acquire a deeper knowledge of the composition of these extracts.

3.2. Total phenolic compounds of the insect extracts

For the additional characterization of the insect extracts, their total phenolic content (TPC) was determined, as these ~~are~~ compounds ~~that~~ have been ~~described~~ ~~reported in for~~ diverse insect species. The TPC of the extracts was performed spectrophotometrically by the Folin-Ciocalteu method. It is important to remark that although this method is known for not being ~~totally-completely~~ specific for phenolic compounds, its purpose in this study was to compare between extracts. Therefore, in order to precisely ~~measure—quantify~~ the amount of total phenolics, more sensitive and specific chromatographic methods would be needed.

As shown in Figure 2, variable values of TPC were obtained for all the extracts, which were within the range of 0.3-5.0 g GAE/100 g of extract, ~~corresponding-being~~ the highest TPC value ~~found in to~~ the UAE-E:W extracts of *A. domesticus*. However, in general, a lack of significant effect of the insect species was obtained ($p = 0.575$), suggesting that the TPC of *A. domesticus* and *T. molitor* extracts ~~were-was~~ similar. ~~Concerning the method of extraction, a significant effect of this factor was not detected either~~ ($p = 0.883$). This suggested that both PLE and UAE caused a similar extraction of TPC, regardless of the insect species and the solvent used. Only the extraction solvent caused significant differences ($p < 0.001$). Thus, all the E:W extracts showed higher TPC values than the E extracts (mean values of 3.8 ± 0.8 g GAE/100 g and 0.8 ± 0.4 g GAE/100 g, respectively). This result was expected, since the higher polarity of the E:W mixture compared with E might allow the extraction of a wider range of compounds of different polarity, as many phenolic compounds might be.

Several previous studies have described the presence of phenolic compounds in insects, but the available information for *A. domesticus* or *T. molitor* is scarce. Some authors detected phenolic compounds in the cuticle or the secretions from the defensive glands of insects belonging to the family of *T. molitor* (Coleoptera) (Andersen, 2010; Tschinkel, 1969). Musundire et al. (2014) reported a TPC of 0.8 g GAE/100 g of extract for the insect *Henicus whellani* (Orthoptera) extracted by Soxhlet with petroleum ether. Such value was similar to those found in the present

study for both PLE-E extracts. Additionally, Liu et al. (2012) described a value of 5 g GAE/100 g of the extract from the insect *Holotrichia parallela* (Coleoptera) obtained by microwave assisted extraction with E:W. This value was closer to the UAE-E:W extracts obtained in the present study. Therefore, all the assayed extracts contained relevant amounts of total phenolics, but variable, mainly due to the extraction solvent, being aqueous ethanol preferred for insect extracts richer in phenolics. Taking into account these preliminary results, further studies would be necessary to precisely quantify the total amount of phenolic compounds by more sensitive chromatographic techniques and confirm the observed differences in the extraction of TPC from the different insect extracts.

331

3.3. Antioxidant activity of the insect extracts

The potential antioxidant activity of the extracts was evaluated by the assay of the ability of the extracts to inhibit the DPPH \cdot radical. All the extracts showed antioxidant activity, most of them causing an inhibitory activity closer to 80%, as shown in Figure 3.a. In general, it seemed that the extracts from *A. domesticus* were more effective than those from *T. molitor* (mean values around 72% and 57% and, respectively), regardless of the method of extraction and the solvent. However, these differences were not significant ($p = 0.346$), suggesting that extracts from both insects have similar antioxidant activity. ~~Concerning the method of extraction, this factor did not caused significant differences on the antioxidant activity of the extracts,~~ No significant differences were observed on the antioxidant activity of the extracts when considering the method of extraction, regardless of the insect species or the solvent ($p = 0.121$). Finally, only the extraction solvent significantly affected the antioxidant values ($p = 0.001$). Thus, those extracts obtained by E:W were more efficient compared to E (mean values around 86% and 44%, respectively), regardless of the method of extraction and the insect species. Considering that these significant differences due to the extraction solvent were also obtained for the TPC values, and that the antioxidant activity is frequently related to the TPC, a correlation study was performed between both variables. A

348 significant positive correlation was found (r Pearson = 0.786; p < 0.001). Thus, as shown in Figure
349 3.b, which illustrates the change of DPPH inhibition according to the TPC values, it seemed that the
350 highest the TPC value, the highest the DPPH \cdot inhibition. This effect was observed for TPC values
351 under 2 g GAE/100 g extract, since above that value, the DPPH \cdot inhibition seemed to reach a
352 plateau closer to 90% and was not affected by the concentration of TPC. This was an interesting
353 result since, as far to our knowledge, previous data about this particular relationship between DPPH
354 and TPC has not been found.

355 In agreement with the obtained results, Liu et al. (2012) stated that ~~the~~ phenolic compounds were
356 responsible for the antioxidant activity of the ethanolic extracts from the insect *Holotrichia*
357 *parallela* (Coleoptera), whereas in the case of the E:W extracts from the same insect, these authors
358 related the antioxidant activity to the proteins of the extracts. Similarly, Zielińska et al. (2017)
359 described the antioxidant potential of hydrolyzed extracts of proteins from edible insects. In this
360 sense, taking into account the qualitative composition of the extracts previously described (Tables 1
361 and 2), correlation studies were also performed between the DPPH values of the extracts and the
362 total chromatographic area of each detected group of chemical compounds. A lack of significant
363 correlation was found between the DPPH \cdot inhibition and abundance of each of the different 12
364 groups of compounds. Therefore, despite the correlation found between TPC and DPPH values in
365 the present study, further studies would be necessary in order to more specifically identify the
366 compounds responsible for the antioxidant activity of the assayed extracts.

367

368 **3.4. Inhibitory activity of pancreatic lipase by the insect extracts**

369 The inhibition of the main enzyme responsible for the digestion of dietary lipids, namely pancreatic
370 lipase, is a potential strategy that is used against pathologies related to the metabolism of lipids,
371 such as obesity, overweight, hypertriglyceridemia or hypercholesterolemia. Diverse bioactive
372 compounds found in natural sources have been linked to this inhibitory activity, such as
373 polyphenols, saponins, terpenes, aminoacids, carotenoids or chitosan (Birari & Bhutani, 2007; de la

374 Garza, Milagro, Boque, Campión, & Martínez, 2011). Therefore, taking into account the presence
375 of phenolic compounds in the insect extracts, and the potential presence of other bioactive
376 compounds described for insects, such as saponins (Musundire et al., 2014; Zhang, Haga,
377 Sekiguchi, & Hirano, 2000), the evaluation of the inhibitory activity of insect extracts was
378 considered of interest. This is because, additionally, evidences suggest that some insect extracts
379 might be bioactive in the metabolism of lipids, although by a non-elucidated mechanism. Thus, *in*
380 *vivo* studies in mice showed antiadipogenic and antiobesity effects when fed with aqueous solutions
381 of the insects *T. molitor* and *Allomyrina dichotoma* (Seo et al., 2017; Yoon et al., 2015); whilst Ali
382 & Arumugam (2011) described an improvement of hypercholesterolemia and atherosclerosis in
383 rabbits fed extracts of *Bombyx mori* cocoons.

384 As shown in Figure 4, all the insect extracts inhibited the activity of the pancreatic lipase and the
385 IC₅₀ values varied in the range of 0.15 to 0.91 mg extract/mL. A significant effect due to the insect
386 species was found ($p = 0.047$). Thus, regardless of the method of extraction and the solvent, ~~those~~
387 extracts from *T. molitor* were significantly more effective than those from *A. domesticus* (mean IC₅₀
388 values around 0.4 mg/mL and 0.7 mg/mL, respectively). Additionally, regardless of the insect
389 species and the solvent, the method of extraction also affected the inhibitory activity **significantly** (p
390 $= 0.001$). Thus, the PLE extracts were more effective for the inhibition of pancreatic lipase than the
391 UAE extracts (mean IC₅₀ values around 0.4 mg/mL and 0.7 mg/mL, respectively). This would
392 suggest that PLE conditions might allow the concentration of inhibitory compounds from the
393 assayed insects in a more effective way. In fact, the strongest IC₅₀ values corresponded to both
394 PLE-E:W extracts for both insect species (0.26 mg/mL for *A. domesticus* and 0.15 mg/mL for *T.*
395 *molitor*). On the contrary, the solvent of extraction did not affect the inhibitory activity of the
396 extracts ($p = 0.329$).

397 The results in the present study were of great interest since, as far as we know, this is the first time
398 that the inhibitory activity against pancreatic lipase achieved by insects has been described.
399 Additionally, the obtained IC₅₀ values could be considered quite valuable and even comparable to

other natural extracts from different plants, which are being frequently explored for such bioactivity. As an example, an IC₅₀ value of 0.26 mg/mL was described for *Chamomilla recutita*, 0.12 mg/mL for *Echinodorus grandiflorus* or 0.15 mg/mL for *Salvia spinose* (Franco et al., 2018; Saad, Zaid, Shanak, & Kadan, 2017), which are similar to those found for the PLE-E:W extracts of insects obtained in the present study. Additionally, higher IC₅₀ values can also be found for other plants, such as 0.76 mg/mL for extracts from *Centella asiatica* (Supkamonseni, Thinkratok, Meksuriyen, & Srisawat, 2014), or up to 7.85 mg/mL for extracts from *Menta spicata* or 7.00 mg/mL for extracts from *Rosmarinus officinalis* (Saad et al., 2017).

The identification of the specific compounds related to the inhibitory activity against pancreatic lipase from these insect extracts is complicated, but similarly to the DPPH activity, correlation studies were performed between the IC₅₀ values and the total chromatographic abundance of the different chemical groups contained in the extracts. A lack of relationship was found between both variables. Therefore, further studies would be necessary in order to elucidate the specific compounds found in insect extracts of this nature that might be responsible for the inhibitory activity observed.

4. CONCLUSIONS

Extracts from the edible insects *Acheta domesticus* and *Tenebrio molitor* can be obtained by advanced methods of extraction such as PLE or UAE, allowing the enrichment in a wide diversity of chemical compounds, such as free fatty acids, aminoacids, organic acids or carbohydrates, as well as in other potential bioactive compounds of interest that might be worth to characterize. In fact, all the extracts show multifunctional bioactivity, such as antioxidant and inhibition of pancreatic lipase enzyme, the latter being described for the first time for insects.

Concerning the antioxidant activity, both insect species show similar bioactive interest, but the solvent of extraction is the most relevant factor to obtain insect extracts with a more efficient antioxidant activity, being more polar solvents such as aqueous ethanol preferred over ethanol. On

426 the contrary, the method of extraction is the most relevant factor to obtain insect extracts with more
427 efficient inhibitory activity against pancreatic lipase, being the PLE method preferred. Additionally,
428 the insect *Tenebrio molitor* would be more effective for such inhibitory activity compared with
429 *Acheta domesticus*.

430 This study shows that insect extracts might be an additional way to impulse other alternative
431 presentations of insect-based foods for human consumption and to provide an added value to the
432 edible insects industry by the production of bioactive ingredients for nutraceutical or food purposes.

433

434 **ABBREVIATIONS USED**

E	Ethanol
E:W	Ethanol:Water
GAE	Gallic Acid Equivalents
PLE	Pressurized liquid extraction
TPC	Total Phenolics Content
UAE	Ultrasound-assisted extraction

435

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442

443 **Notes**

444 Declaration of interests: none.

445

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576 **Figure Captions**

577

578 **Figure 1.** Total chromatographic area of major chemical groups detected by GC-MS for the insect
579 extracts.

580

581 **Figure 2.** TPC of insect extracts (g GAE/100 g extract). Different letters between extracts are
582 significantly different ($p \leq 0.05$).

583

584 **Figure 3.** Antioxidant activity of insect extracts. a) % of inhibited DPPH \cdot and b) ~~Correlation~~
585 ~~between the~~ DPPH \cdot inhibition (%) ~~versus and~~ the TPC value (g GAE/100 g extracts) of the insect
586 extracts. *T. molitor*: PLE-E, ■; PLE-E:W, ▲; UAE-E, ●; UAE-E:W, ◆. *A. domesticus*: PLE-E, □;
587 PLE-E:W, △; UAE-E, ○; UAE-E:W, ◇. Different letters between extracts are significantly different
588 ($p \leq 0.05$).

589

590 **Figure 4.** Inhibitory activity of pancreatic lipase by insect extracts (IC₅₀ value, mg extract/mL).
591 Different letters between extracts are significantly different ($p \leq 0.05$).

Table 1. GC-MS characterization of *A. domesticus* extracts

Table 1. GC-MS characterization of <i>A. domestica</i> extracts									
t _R	Compound	PLE				UAE			
		E:W		E		E:W		E	
		Area	%	Area	%	Area	%	Area	%
LIPIDS									
	Fatty acids	196426013	55.16	245372831	83.27	33332023	9.00	289908774	84.50
10.00	Butanedioic acid	914318	0.26	612980	0.21	1536838	0.41	418548	0.12
12.73	Dodecanoic acid	413586	0.12	442657	0.15	-	-	522096	0.15
14.13	Tetradecanoic acid	2686565	0.75	2488245	0.84	1688155	0.46	3193969	0.93
15.07	Hexadecanoic acid EE*	1078247	0.30	717406	0.24	-	-	1186763	0.35
15.30	cis-9-Hexadecenoic acid	3416392	0.96	4181050	1.42	-	-	4790926	1.40
15.51	Hexadecanoic acid	60759213	17.06	80814736	27.43	7907486	2.13	94541063	27.56
16.01	Heptadecanoic acid	-	-	546623	0.19	-	-	645772	0.19
16.12	9,12-Octadecadienoic acid EE*	-	-	-	-	-	-	2562233	0.75
16.15	Ethyl Oleate	4307080	1.21	2222729	0.75	-	-	1893912	0.55
16.56	9,12-Octadecadienoic acid	107483711	30.18	134495436	45.64	20476435	5.53	157693786	45.96
16.65	Octadecanoic acid	14995536	4.21	18274896	6.20	1723109	0.47	21207292	6.18
17.54	11-Eicosenoic acid	-	-	-	-	-	-	701909	0.20
17.66	Eicosanoic acid	371365	0.10	576073	0.20	-	-	550505	0.16
	Glycerides	10074731	2.83	8509840	2.89	4035515	1.09	12076168	3.52
18.29	Monoglyceride n.i.	-	-	-	-	-	-	559284	0.16
18.47	Monoglyceride n.i.	1739067	0.49	1143402	0.39	-	-	2132554	0.62
18.50	Monoglyceride n.i.	827117	0.23	748346	0.25	-	-	721080	0.21
19.11	Monoglyceride n.i.	590415	0.17	399477	0.14	-	-	601703	0.18
19.30	Monoglyceride n.i.	2642813	0.74	2735459	0.93	1159752	0.31	4038741	1.18
19.39	Monoglyceride n.i.	652890	0.18	-	-	1375813	0.37	432674	0.13
27.97	Diglyceride n.i.	130342	0.04	144529	0.05	-	-	299388	0.09
28.65	Diglyceride n.i.	123561	0.03	346012	0.12	-	-	157494	0.05
31.46	Diglyceride n.i.	745060	0.21	709027	0.24	374515	0.10	1032000	0.30
32.41	Diglyceride n.i.	745862	0.21	546168	0.19	240806	0.07	632543	0.18
36.28	Diglyceride n.i.	1004576	0.28	917246	0.31	544378	0.15	779431	0.23
37.69	Diglyceride n.i.	873028	0.25	820174	0.28	340251	0.09	689276	0.20
NITROGEN COMPOUNDS									
	Amino acids and derivatives	36126812	10.14	16375986	5.56	93286325	25.18	22830975	6.65
8.17	Glycine	4446130	1.25	1107677	0.38	14316594	3.86	2245541	0.65
8.68	l-Proline	836592	0.23	-	-	-	-	-	-
9.13	L-Valine	1184357	0.33	1941235	0.66	6150396	1.66	3662484	1.07
9.66	L-Leucine	1650142	0.46	1057830	0.36	5288245	1.43	3585919	1.05
9.88	L-Proline	2589045	0.73	3177353	1.08	9643360	2.60	5467930	1.59
10.46	Serine	2638354	0.74	573469	0.19	6458728	1.74	651175	0.19
10.70	L-threonine	1075545	0.30	573280	0.19	2998750	0.81	629116	0.18
11.84	Pyroglutamic acid	7315815	2.05	4204826	1.43	8957783	2.42	2091094	0.61

Table 1. GC-MS characterization of *A. domesticus* extracts (continued)

t _R	Compound	PLE				UAE			
		E:H		E		E:H		E	
		Area	%	Area	%	Area	%	Area	%
12.52	Ornithine	3218817	0.90	-	-	9655571	2.61	381754	0.11
12.63	Phenylalanine	801132	0.22	473194	0.16	1874332	0.51	1125649	0.33
12.97	L-Asparagine	584171	0.16	-	-	1183037	0.32	-	-
13.14	Amino acid n.i.	-	-	-	-	1742710	0.47	-	-
13.21	Lysine	3261541	0.92	-	-	7924770	2.14	502318	0.15
13.72	Amino acid n.i.	1872558	0.53	1676300	0.57	7718023	2.08	-	-
14.73	Histidine	1966184	0.55	344395	0.12	3867280	1.04	947558	0.28
14.84	L-Tyrosine	2686429	0.75	1246427	0.42	5506746	1.49	1540437	0.45
	Non-protein compounds	36064220	10.13	1845126	0.63	32145921	8.68	2333575	0.68
9.40	Urea	756775	0.21	818970	0.28	-	-	676039	0.20
10.25	Pyrimidine	558790	0.16	757276	0.26	924870	0.25	734103	0.21
11.26	2-Piperidone	2329769	0.65	268880	0.09	6996198	1.89	598066	0.17
11.64	2,4(1H,3H)-Pyrimidinedione	457260	0.13	-	-	1076735	0.29	-	-
13.92	9H-Purine	-	-	-	-	2775228	0.75	325367	0.09
15.93	Uric acid	31961626	8.98	-	-	15429493	4.17	-	-
18.52	Inosine	-	-	-	-	4943397	1.33	-	-
	ACIDS								
	Organic acids	49184327	13.81	14460469	4.91	153775582	41.51	2531214	0.74
10.21	Glyceric acid	595568	0.17	-	-	872274	0.24	-	-
11.56	Malic acid	3299337	0.93	393133	0.13	7138447	1.93	-	-
12.21	α -Hydroxyglutaric acid	-	-	-	-	818644	0.22	-	-
14.07	Citric acid	3932351	1.10	-	-	10120160	2.73	916352	0.27
14.54	d-(+)-Gluconic acid δ -lactone	5896856	1.66	1684601	0.57	13293004	3.59	486384	0.14
14.70	L-Gluconic acid lactone	11010984	3.09	5906174	2.00	47048774	12.70	-	-
15.36	D-Gluconic acid	24449231	6.87	6476561	2.20	73792803	19.92	1128478	0.33
16.05	2-Keto-d-gluconic acid	-	-	-	-	691476	0.19	-	-
	Inorganic acids	-	-	-	-	-	-	1993250	0.58
13.67	Phosphoric acid	-	-	-	-	-	-	1993250	0.58
	CARBOHYDRATES								
	Sugars	24311728	6.83	2137099	0.73	50531566	13.64	5081000	1.48
13.94	Glucufuranoside	2779858	0.78	-	-	-	-	-	-
14.02	D-Fructose	3360485	0.94	-	-	9076034	2.45	705890	0.21
14.24	D-Xylose	1310206	0.37	-	-	-	-	-	-
14.29	β -D-Galactofuranose	6288643	1.77	-	-	7700699	2.08	-	-
14.63	Monosaccharide n.i.	-	-	-	-	-	-	1881593	0.55

Table 1. GC-MS characterization of *A. domesticus* extracts (continued)

t _R	Compound	PLE				UAE			
		E:H		E		E:H		E	
		Area	%	Area	%	Area	%	Area	%
14.72	Monosaccharide n.i.	1896692	0.53	448743	0.15	-	-	-	-
15.18	Monosaccharide n.i.	5623513	1.58	1393490	0.47	26591542	7.18	2323476	0.68
18.78	Monosaccharide n.i.	444777	0.12	-	-	873820	0.24	-	-
19.03	Monosaccharide n.i.	967367	0.27	-	-	1299619	0.35	-	-
19.16	Monosaccharide n.i.	579401	0.16	-	-	999766	0.27	-	-
19.34	Monosaccharide n.i.	-	-	-	-	930565	0.25	-	-
19.48	Monosaccharide n.i.	-	-	294866	0.10	1479558	0.40	170041	0.05
19.52	Monosaccharide n.i.	1060786	0.30	-	-	1579963	0.43	-	-
	Sugar alcohols	604669	0.17	1902211	0.65	3305258	0.89	984480	0.29
13.45	d-(+)-Arabitol	604669	0.17	1473545	0.50	2027813	0.55	653957	0.19
14.22	Myo-Inositol	-	-	428666	0.15	1277445	0.34	330523	0.10
STEROLS									
21.49	Cholecalciferol	396239	0.11	348935	0.12	-	-	290587	0.08
21.27	Cholesterol	1175109	0.33	1755414	0.60	38412	0.01	1161057	0.34
	Phytosterols	181896	0.05	312063	0.11	-	-	357073	0.10
21.59	Lanosterol	78589	0.02	140256	0.05	-	-	127018	0.04
21.88	Campesterol	49437	0.01	74015	0.03	-	-	117070	0.03
22.44	β-Sitosterol	53870	0.02	97792	0.03	-	-	112985	0.03
HYDROCARBONS									
	Alkanes*	1559562	0.44	1643884	0.56	-	-	3544809	1.03
19.71	Alkane n.i.	297349	0.08	350412	0.12	-	-	510119	0.15
19.88	Alkane n.i.	177683	0.05	-	-	-	-	224039	0.07
20.02	Alkane n.i.	190143	0.05	-	-	-	-	247384	0.07
20.60	Alkane n.i.	276089	0.08	345306	0.12	-	-	473182	0.14
20.92	Alkane n.i.	177021	0.05	-	-	-	-	315560	0.09
22.03	Alkane n.i.	183398	0.05	289383	0.10	-	-	395945	0.12
22.19	Alkane n.i.	-	-	-	-	-	-	102396	0.03
22.70	Alkane n.i.	-	-	78206	0.03	-	-	136156	0.04
22.90	Alkane n.i.	-	-	86707	0.03	-	-	132019	0.04
23.50	Alkane n.i.	-	-	210247	0.07	-	-	320194	0.09
23.71	Alkane n.i.	257879	0.07	283623	0.10	-	-	401561	0.12
24.89	Alkane n.i.	-	-	-	-	-	-	286254	0.08

All the compounds were found under their trimethylsilyl derivative form except those marked with *.

EE = ethyl ester; n.i. = non identified

Table 2. GC-MS Characterization of *Tenebrio molitor* extracts.

Table 2. GC-MS Characterization of <i>Tenebrio molitor</i> Extracts.									
t _R	Compound	PLE				UAE			
		E:W		E		E:W		E	
		Area	%	Area	%	Area	%	Area	%
LIPIDS									
	Fatty acids	128143400	73.90	220240280	79.10	40658583	10.10	121041340	84.60
8.65	Butanoic acid	-	-	-	-	1009931	0.25	-	-
9.30	Pentanoic acid	-	-	-	-	-	-	100144	0.07
9.31	2-Hydroxyisocaproic acid	-	-	-	-	543245	0.14	-	-
10.00	Butanedioic acid	143046	0.08	231734	0.08	1137339	0.28	-	-
10.19	Propanoic acid	1254229	0.73	-	-	-	-	-	-
12.73	Dodecanoic acid	615724	0.36	1048460	0.38	692982	0.17	642443	0.45
13.94	9-Tetradecenoic acid	-	-	-	-	-	-	386833	0.27
14.13	Tetradecanoic acid	6330679	3.68	10490475	3.79	4624381	1.15	5523657	3.88
14.77	n-Pentadecanoic acid	329515	0.19	546214	0.20	-	-	260016	0.18
15.07	Hexadecanoic acid EE*	935059	0.54	-	-	-	-	84273	0.06
15.30	cis-9-Hexadecenoic acid	3722959	2.17	4293956	1.55	-	-	3086396	2.17
15.51	Hexadecanoic acid	26628961	15.49	48228373	17.44	8047414	2.01	24838707	17.45
15.86	cis-10-Heptadecenoic acid	-	-	-	-	-	-	308992	0.22
16.01	Heptadecanoic acid	282558	0.16	517366	0.19	-	-	318373	0.22
16.15	Ethyl Oleate	2791507	1.62	521283	0.19	-	-	317821	0.22
16.56	9,12-Octadecadienoic acid	77741821	45.21	140805243	50.91	14762255	3.68	78587553	55.20
16.65	Octadecanoic acid	5618637	3.27	10647593	3.85	9841036	2.45	4960065	3.48
17.00	cis-10-Nonadecenoic acid	-	-	-	-	-	-	114758	0.08
17.15	Nonadecanoic acid	-	-	-	-	-	-	107815	0.08
17.49	Myristic acid	-	-	-	-	-	-	136722	0.10
17.54	11-Eicosenoic acid	590837	0.34	1065371	0.39	-	-	561691	0.39
17.66	Eicosanoic acid	162163	0.09	291338	0.11	-	-	160645	0.11
	Glycerides	7507049	4.37	11016177	3.98	3749005	0.93	8008061	5.63
17.32	Monoglyceride n.i.	-	-	-	-	-	-	54784	0.04
18.47	Monoglyceride n.i.	995705	0.58	1552874	0.56	-	-	544436	0.38
19.11	Monoglyceride n.i.	334690	0.19	349398	0.13	-	-	354649	0.25
19.30	Monoglyceride n.i.	1584000	0.92	2914712	1.05	1347194	0.34	1823604	1.28
19.39	Monoglyceride n.i.	281038	0.16	106864	0.04	883604	0.22	130968	0.09
27.68	Diglyceride n.i.	138413	0.08	197418	0.07	-	-	109652	0.08
27.97	Diglyceride n.i.	92498	0.05	102177	0.04	-	-	68703	0.05
28.35	Diglyceride n.i.	243062	0.14	359304	0.13	-	-	191530	0.13
28.65	Diglyceride n.i.	93707	0.05	107807	0.04	-	-	69645	0.05
31.46	Diglyceride n.i.	798897	0.46	1154533	0.42	393886	0.10	1062786	0.75
32.41	Diglyceride n.i.	931988	0.54	1179210	0.43	195897	0.05	844430	0.59
36.28	Diglyceride n.i.	1438547	0.84	2345527	0.85	559304	0.14	1816967	1.28
37.69	Diglyceride n.i.	1570209	0.91	2199227	0.80	369120	0.09	1480343	1.04

Table 2. GC-MS Characterization of *Tenebrio molitor* extracts (continued)

t _R	Compound	PLE				UAE			
		E:H		E		E:H		E	
		Area	%	Area	%	Area	%	Area	%
NITROGEN COMPOUNDS									
	Amino acids and derivatives	20385213	11.86	27851306	10.07	214864698	53.55	7685315	5.40
8.17	Glycine	905188	0.53	645454	0.23	10032982	2.50	-	-
8.50	I-Isoleucine	364712	0.21	-	-	-	-	481309	0.34
8.68	I-Proline	718701	0.42	-	-	3308706	0.82	1145171	0.80
8.89	β-Alanine	80727	0.05	-	-	628375	0.16	-	-
9.13	L-Valine	2013802	1.17	4681135	1.69	20005868	4.99	974530	0.68
9.88	L-Proline	4055480	2.36	9993905	3.61	42429191	10.58	2469068	1.73
10.46	Serine	1069423	0.62	774616	0.28	9683654	2.41	75776	0.05
10.70	L-threonine	755463	0.44	1051317	0.38	5918312	1.48	170414	0.12
10.97	I-Aspartic acid	64316	0.04	-	-	580039	0.14	-	-
11.84	Pyroglutamic acid	4170771	2.43	7029712	2.54	30144859	7.51	1368407	0.96
11.97	Alanine	132060	0.08	329628	0.12	800583	0.20	287530	0.20
12.52	Ornithine	192728	0.11	-	-	8771757	2.19	-	-
12.56	Glutamine	2296933	1.34	324520	0.12	15974659	3.98	98420	0.07
12.63	Phenylalanine	815258	0.47	1378109	0.50	8518431	2.12	476611	0.33
12.71	Phenylethanolamine	-	-	-	-	602844	0.15	-	-
12.90	Homocysteine	-	-	-	-	724636	0.18	-	-
13.21	Lysine	1100772	0.64	-	-	15005128	3.74	-	-
13.76	I-Glutamine	-	-	-	-	7825712	1.95	-	-
14.45	Tyrosine	-	-	192709	0.07	-	-	138079	0.10
14.73	Histidine	1033712	0.60	654125	0.24	16919696	4.22	-	-
14.84	L-Tyrosine	615167	0.36	796076	0.29	8328989	2.08	-	-
16.61	L-Tryptophan	-	-	-	-	8660277	2.16	-	-
	Non-protein compounds	4066546	2.37	4287734	1.55	40107877	10.00	544836	0.38
9.40	Urea	671740	0.39	1144261	0.41	-	-	316769	0.22
10.25	Pyrimidine	225291	0.13	769260	0.28	-	-	228067	0.16
11.26	2-Piperidone	433695	0.25	356867	0.13	5175645	1.29	-	-
13.92	9H-Purine	-	-	836649	0.30	3380222	0.84	-	-
15.82	Pyrrolidine	261012	0.15	-	-	1634251	0.41	-	-
15.93	Uric acid	2474808	1.44	1180697	0.43	24493924	6.11	-	-
17.85	Uridine	-	-	-	-	1540812	0.38	-	-
18.52	Inosine	-	-	-	-	3883023	0.97	-	-
ACIDS									
	Organic acids	2450623	1.43	2355016	0.85	30232501	7.54	705095	0.50
10.21	Glyceric acid	-	-	-	-	10357207	2.58	-	-
11.56	Malic acid	402817	0.23	-	-	1419856	0.35	119930	0.08
12.21	α-Hydroxyglutaric acid	441444	0.26	163444	0.06	4164058	1.04	119772	0.08

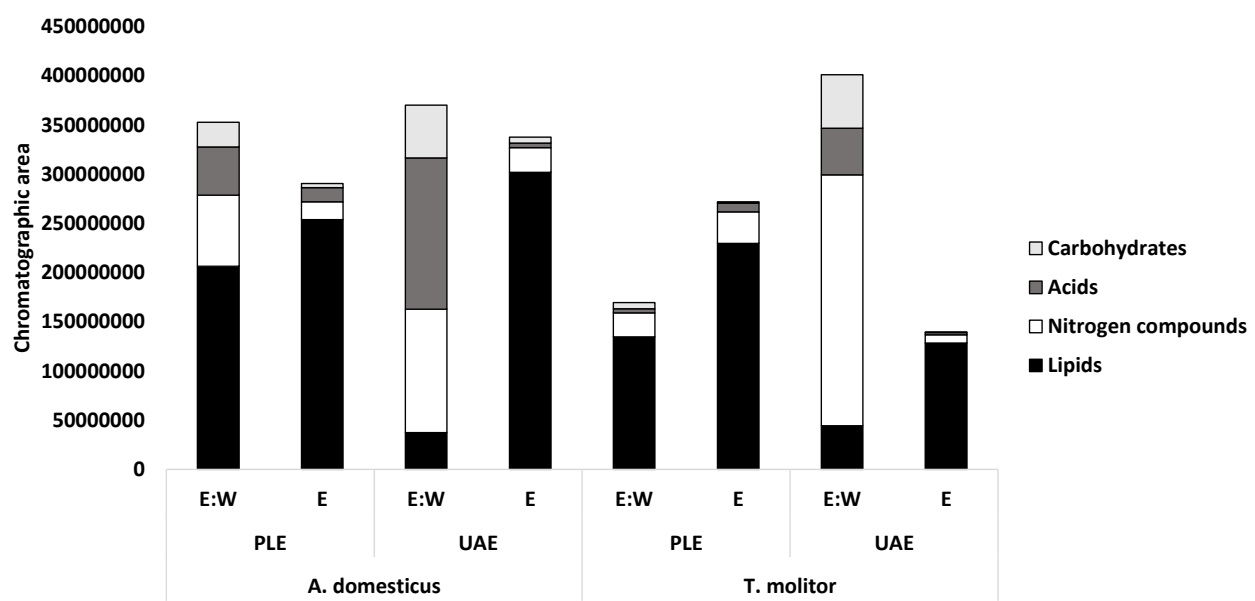
Table 2. GC-MS Characterization of *Tenebrio molitor* extracts (continued)

t _R	Compound	PLE				UAE			
		E:H		E		E:H		E	
		Area	%	Area	%	Area	%	Area	%
14.07	Citric acid	451808	0.26	-	-	4394035	1.10	-	-
14.70	L-Gluconic acid lactone	-	-	710357	0.26	-	-	-	-
15.36	D-Gluconic acid	790291	0.46	545194	0.20	9897345	2.47	-	-
17.60	Sebacic acid	364263	0.21	936021	0.34	-	-	465393	0.33
	Inorganic acids	1662409	0.97	6407763	2.32	17273521	4.31	1656578	1.16
12.39	Phosphoric acid derivative n.i.	-	-	652597	0.24	-	-	469442	0.33
13.67	Phosphoric acid	1160181	0.67	5251931	1.90	10056746	2.51	1187136	0.83
15.61	Phosphoric acid derivative n.i.	502228	0.29	503235	0.18	7216775	1.80	-	-
CARBOHYDRATES									
	Sugars	6418498	3.73	599771	0.22	52961901	13.20	444012	0.31
13.94	Glucofuranoside	1128956	0.66	-	-	-	-	-	-
14.02	D-Fructose	-	-	-	-	2127673	0.53	-	-
14.29	β-D-Galactofuranose	2257916	1.31	-	-	3236607	0.81	-	-
14.63	Monosaccharide n.i.	1290428	0.75	-	-	15918476	3.97	271924	0.19
14.72	Monosaccharide n.i.	576722	0.34	-	-	-	-	-	-
15.18	Monosaccharide n.i.	1164476	0.68	599771	0.22	24846369	6.19	172088	0.12
15.29	Monosaccharide n.i.	-	-	-	-	3273009	0.82	-	-
16.75	Monosaccharide n.i.	-	-	-	-	1599968	0.40	-	-
16.94	Monosaccharide n.i.	-	-	-	-	1959799	0.49	-	-
	Sugar alcohols	-	-	925805	0.33	1334794	0.33	-	-
13.45	d-(+)-Arabitol	-	-	925805	0.33	1334794	0.33	-	-
STEROLS									
21.49	Cholecalciferol	371972	0.22	632951	0.23	-	-	341370	0.24
21.27	Cholesterol	378022	0.22	879294	0.32	25075	0.01	499313	0.35
	Phytosterols	37532	0.02	70812	0.03	-	-	94047	0.07
21.88	Campesterol	-	-	-	-	-	-	40008	0.03
22.44	β-Sitosterol	37532	0.02	70812	0.03	-	-	54039	0.04
HYDROCARBONS									
	Alkanes*	1512953	0.88	2860070	1.03	-	-	1882488	1.32
17.95	Alkane n.i.	322616	0.19	558317	0.20	-	-	319777	0.22
19.50	Alkane n.i.	658060	0.38	1284525	0.46	-	-	763735	0.54
19.88	Alkane n.i.	77263	0.04	106837	0.04	-	-	78120	0.05
20.02	Alkane n.i.	147255	0.09	284219	0.10	-	-	217886	0.15
20.19	Alkane n.i.	96669	0.06	182629	0.07	-	-	145340	0.10
20.92	Alkane n.i.	105368	0.06	201635	0.07	-	-	160756	0.11
21.07	Alkane n.i.	105722	0.06	176620	0.06	-	-	140191	0.10
22.03	Alkane n.i.	-	-	65288	0.02	-	-	56683	0.04

All the compounds were found under their trimethylsilyl derivative form except those marked with *.

EE = ethyl ester; n.i. = non identified

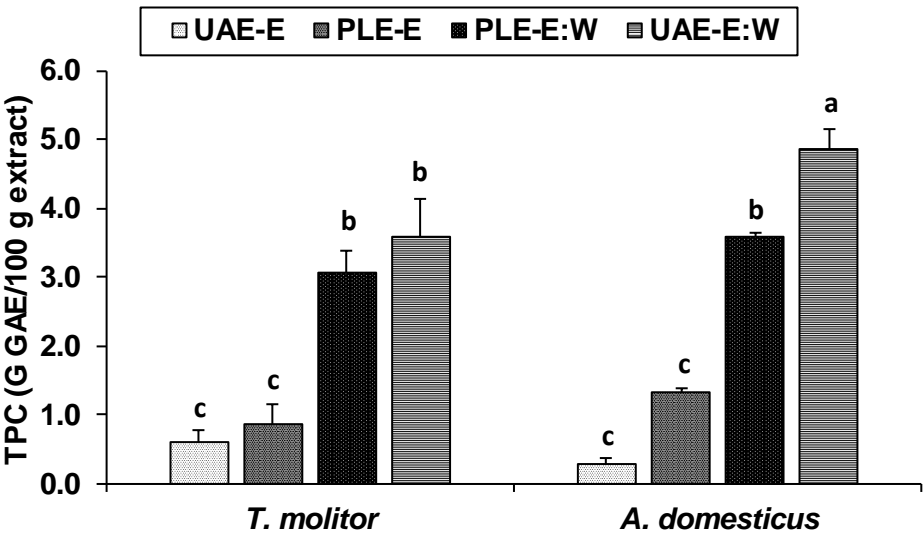
592 **Figure 1.**



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595 **Figure 2.**



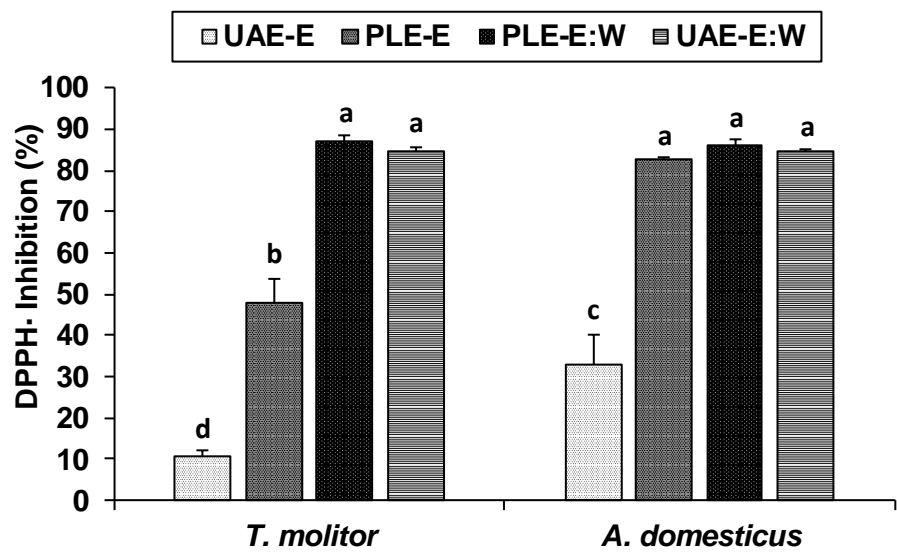
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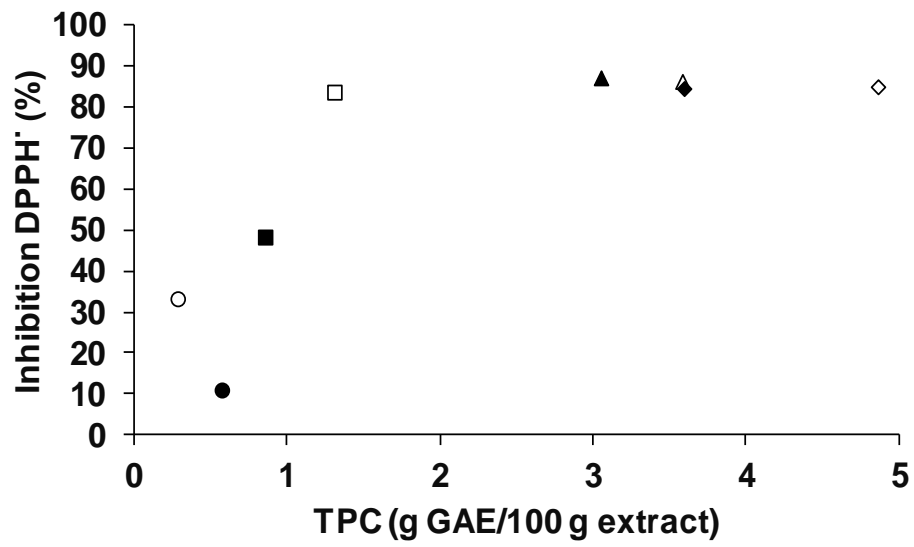
599 **Figure 3.**

600 **a)**



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602 **b)**

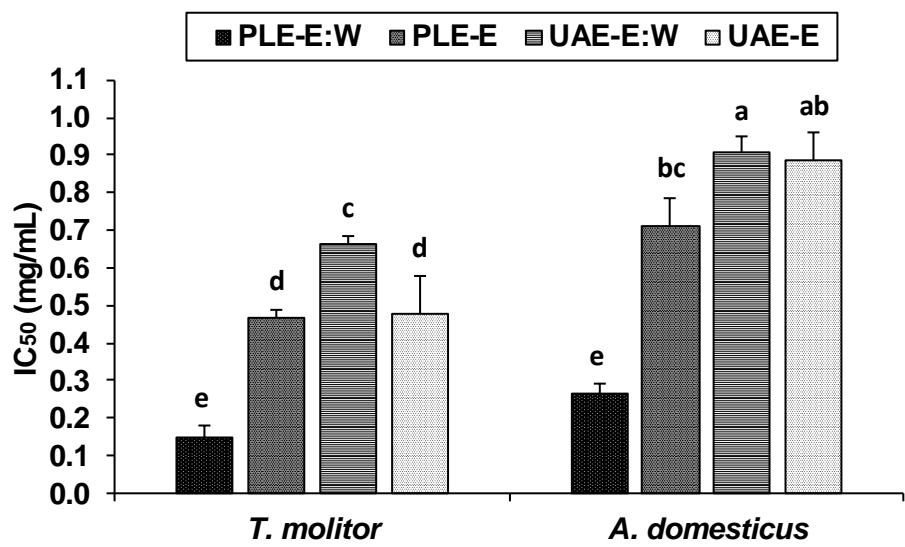


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607 **Figure 4.**



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