Heliyon 8 (2022) e09455

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Characterization of lipid extracts from the *Hermetia illucens* larvae and their bioactivities for potential use as pharmaceutical and cosmetic ingredients

Cíntia Almeida [a,b], Daniel Murta [c,d,e], Rui Nunes [c], André Rolim Baby [f], Ângela Fernandes [g], Lillian Barros [g], Patricia Rijo [a,*,1], Catarina Rosado [a,1]

[a] Universidade Lusófona (CBIOS – Research Center for Biosciences & Health Technologies), Lisboa, Portugal
[b] Department of Biomedical Sciences, University of Alcalá, Ctra, Madrid-Barcelona, Km 33.600, Alcalá de Henares, 28871, Madrid, Spain
[c] Ingredient Odyssey SA – EntoGreen, Rua Cidade de Santarém 140, 2005-079 Santarém, Portugal
[d] CiiEM – Centro de Investigação Interdisciplinar Egas Moniz, Campus Universitário, 2829-511 Caparica, Portugal
[e] Myrtus Unipessoal Lda, Monte Claro, Nisa, Portugal
[f] Faculty of Pharmaceutical Sciences, Universidade de São Paulo, São Paulo, Brazil
[g] Centro de Investigação da Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

ARTICLE INFO

Keywords:
Insect
Hermetia illucens
Lipids
Biomass
Skin

ABSTRACT

There is an increasingly growing demand for the use of natural and sustainable bioactives in the field of the pharmaceutical and cosmetic industries. The biomass from black soldier fly larvae (*Hermetia illucens*) can be viewed as an innovative source of compounds with high aggregate value and marketing potential due to the sustainable organic matter bioconversion process used as substrate for its development. This insect can be a source of lipid compounds with high added value, mainly due to its high content in fatty acids (FA) with potential applicability in the pharmaceutical and cosmetic industry. In this context, in this work different extraction methods were tested (decoction, microwaves, maceration and ultrasound), using water, acetone, *n*-hexane as extraction solvents, to evaluate yields of the BSF larvae lipid extracts, as well as their lipid profile, and a preliminary safety screening was conducted. Results show that despite using different extraction techniques and solvents, similar FA composition profiles were obtained. The lauric acid content (C12: 0) is elevated in all the extracts in relation to the other FA, ranging 37%–62%. The contents in palmitic (C16: 0) and oleic (C18: 1n-9) acids, were also high in all applied extraction methods. The omega-6 FA (ω-6 PUFAs), mainly linoleic acid (C18: 2n6c), were also identified in the lipid fraction of BSF larvae biomass, with a content variation between 4.5% and 17.7%, while the omega-3 group, namely α-Linolenic acid (C18: 3n3), presented values between 0.66% and 1.95%. None of the extracts presented toxicity in preliminary tests with the *Artemia salina* model. Through this study, it was possible to confirm that BSF larvae oil can be obtained by sustainable methods, containing a broad mixture of FA and being highly rich in lauric acid, with a promising skin care applicability.

1. Introduction

The search for sustainable natural source raw materials is a big challenge for the pharmaceutical and cosmetics sector. Natural origin ingredients that offer safety and good performance of their bioactives were already in high demand. However, this trial has recently become more demanding since sustainability should also be added to their requisites.

Current world governmental policies are guided by the United Nations's sustainable development goals of 2030, directed toward an economy of valorization of resources, materials, and products, in order to reduce the accumulation of waste. Thus, an increasingly important area in Research, Development & Innovation is focused on the exploration of sustainable locally produced biomaterials with biomedical applications. This trend for alternative ingredients currently extends from those that act in the formulation rheology, to preservatives and actives that have better biocompatibility and enhanced performances.

In the context of the biorefinery concept and its contribution to a circular economy, *Hermetia Illucens* - Black soldier fly (BSF) - is considered one of the insect species with great potential for large-scale production [1]. The use of BSF larvae as an ingredient for feed and food is already known. However, a significant part of the compounds that can be

* Corresponding author.
E-mail addresses: patricia.rijo@ulusofona.pt, catarina.rosado@ulusofona.pt (P. Rijo).
[1] Shared senior authorship.

https://doi.org/10.1016/j.heliyon.2022.e09455
Received 4 January 2022; Received in revised form 6 March 2022; Accepted 12 May 2022

extracted from BSF larvae biomass has a high applicability potential in the pharmaceutical and cosmetics sector [2, 3], which is a promising market as mentioned above.

The rearing of BSF larvae allows a sustainable organic matter bioconversion process (e.g. food residues) resulting in added-value products [2] since its biomass has a high content in proteins, amino acids, mono and polyunsaturated fatty acids, and polysaccharides such as chitin [4, 5], in addition to micronutrients such as copper, iron, selenium, zinc, and vitamins [6, 7, 8]. As mentioned, the larvae are already used as part of the diet of some animal groups, however, the lipid fraction is often considered inappropriate for consumption due to high content of saturated fatty acids (FA) (*e.g* for fish feed production) and it can become a byproduct of animal feed production [9, 10]. In this sense, an alternative use for this rich fat content present in the larval biomass would be as a source of ingredients for the pharma and cosmetic industry.

The lipid fraction of the BSF larvae biomass is characterized by the high concentration of saturated FA, mainly lauric acid [3, 11, 12]. Interestingly, its lauric acid content is similar to that of coconut oil, which seems to be beneficial to the skin mainly due to this FA [2]. Lauric acid has also some potential as a preservative since it is known to have significant antiviral and antibacterial activities [13]. Other essential FA that are known to be key elements in human skin, mainly in barrier function, are also present in BSF larva oil, such as oleic, palmitic, and linoleic acid [14, 15, 16]. In addition, the blend of FA and their derivatives present in the BSF larvae lipid fraction are often employed as emollients, emulsifiers, and stabilizers of disperse systems in topical and cosmetic formulations [11, 13, 17].

In this context, the present work aimed to further explore the application of BSF larva oil, screening the yields of different biomass extractions, comparing the lipidic compositions of the different extracts and conducting a preliminary assessment of its bioactivity and safety. This study is also focused on efficiently using natural resources, such as insect oil, to ensure more sustainable production of skin formulations.

2. Materials & methods

The different extraction methods were conducted in two cycles, introducing specific modifications aiming to optimize the process and increase yields such as pre-treatment of biomass, extraction solvents, separation technique, or combination of extraction methods [17]. In the first cycle, whole larvae in decoction and microwave methods or ground larvae for the methods of maceration in organic solvents were assayed. In the second cycle, only ground larvae were submitted to all the applied extraction techniques, to improve the extraction process and increase yields, but combinations were attempted, namely, ultrasound and microwaves and ultrasound and organic solvents.

Each lipid extract was fully characterized in terms of composition and antioxidant capacity and a preliminary toxicity assay was conducted (evaluation of general toxicity in the *Artemia salina* model).

2.1. Materials

The larval biomass was provided by the company Entogreen and the larvae were fed the Gainesville diet [18] (50% wheat bran, 30% alfalfa meal, 20% cornmeal) with 70% relative humidity. Briefly, the method described by Almeida *et al.* was followed [13], where 10 g of dried larvae samples were used for each of the extraction methods. The study was divided into two cycles, where different methods of extraction were tested (four methods in the first and five in the second), as explained above.

2.2. Decoction extraction

The biomass larvae were boiled in 100 mL of distilled water for about 3 min [17, 19]. After cooling, the samples were collected and centrifuged (Eppendorf Centrifuge 5804 R, Hamburg, Germany) for 7 min at 5000 rpm at 4 °C. The lipid layer formed at the top, characterized by a pasty and whitish consistency, was collected and heated in a 35–40 °C water bath for 15 min and centrifuged at 1500 x *g* for 30 min at 35 °C [20]. After centrifugation, a transparent lipid fraction was obtained and stored at -40 °C for future analysis.

2.3. Microwave extraction

The larvae samples in 100 mL of distilled water were heated for 30 s at a power of 850 W in a conventional household microwave (Whirlpool Talent, Benton Harbour, MI, USA) [17, 19]. The same procedures (centrifugation and separation of fractions) previously described in the decoction process were then conducted.

2.4. Extraction by maceration with organic solvents

In the extractions by maceration singly two solvents were used, *n*-hexane and acetone. 100 mL of each solvent was added and kept under low agitation for 2 h on a shaking plate (Velp Scientifica-ARE, Usmat, Italy) [17, 21, 22]. After natural sedimentation, the organic supernatant was vacuum filtered using a paper filter (Whatman ø 110mm, Darmstadt, Germany). The solvent was added to the larvae pellet once more and after was removed on a rotary vacuum evaporator (IKA®, Werke RV06-ML, Staufen, Germany) at a temperature of 50 °C. The extract obtained was stored at -40 °C for future analysis.

2.5. Ultrasound extraction combined with microwave

In 100 mL of distilled water the ground larvae mass was first subjected to a 15 min cold ultrasound (VWR model USC-TH, Radner, PA, USA) treatment, followed by heat for 30 s at a power of 850 W in the microwave [17, 19]. The centrifugation and separation processes were the same as described above.

2.6. Ultrasonic extraction with organic solvents

In 100 mL of each solvent (n-hexane and acetone) the ground larvae mass was submitted to a 15 min ultrasound treatment before the timed agitation [17]. The organic supernatant was vacuum filtered using a paper filter resulting in the separation of the organic fraction and the filtrate powder. The solvent was added to the larvae pellet once more and then was removed on a rotary vacuum evaporator (IKA®, Werke RV06-ML, Staufen, Germany) at a temperature of 50 °C. The extract obtained was stored at -40 °C for future analysis.

2.7. Determination of Antioxidant Activity (DPPH)

$$\text{Antioxidant Activity } \% = \left(\frac{Abs\ DPPH - Abssample}{AbsDPPH}\right) x\ 100 \quad (1)$$

Where Abs_{DPPH} refers to the absorbance of DPPH against white and Abs_{sample} refers to the absorbance of samples against white.

The lipid fractions of the extracts obtained were evaluated for their ability to eliminate radicals using 2,2-diphenyl-1-picryl-hydrazil - DPPH, using methods described in the literature [19, 23]. Briefly, 10 μL of each extract sample (previously solubilized in *n*-hexane) was added to a 990 μL solution of DPPH (0.002% in methanol), with a final concentration of 0.01 mg mL^{-1}. The mixture was incubated for 30 min at room temperature and the absorbance was measured at 517 nm against a corresponding blank and the antioxidant activity was calculated (equation 1) [24]. The results were expressed as a percentage of the values obtained by absorbance. Butylated hydroxytoluene - BHT was used as a positive control. Extracts are considered to have antioxidant activity if they can significantly eliminate target radicals. Each assay was performed in triplicate.

2.8. General toxicity bioassay (Artemia salina model)

In order to evaluate the toxicity of the different lipid extracts, a preliminary assay with *A. salina* brine shrimp was performed [25]. *A. salina* model is often used in the biological activity study, as test organisms in ecotoxicology and has broad applications as a research tool for new natural ingredients [26, 27, 28]. The eggs (JBL, Armtio® Pur) were incubated in sterile saline (JBL, Artemio® salt) under constant aeration and lighting for 48 h at 25 °C. After hatching, 900 μL containing around 10 live and active larvae (nauplii) were collected and distributed in each well of the 24-well plate. The extracts of the lipid fractions were solubilized in DSMO at a concentration of 10 mg mL^{-1}. To each well column 100 μL of the saline medium were added (negative control), 100 μL of DMSO (positive control for the solvent of the extracts) and in the remaining wells 100 μL of the samples at the final concentration of 100 μg mL^{-1} (diluted in saline) [24]. After 24 h, the number of dead nauplii was counted. The potassium dichromate compound $K_2Cr_2O_7$ was used as a positive control, as it has high toxicity to *A. salina*. The results were expressed as a percentage (%) of nauplii mortality. Extracts that maintain *A. salina* viability ≥50% are considered non-toxic [25, 29].

2.9. Characterization and quantification of lipid fractions

To assess the FA content a previously established methodology was performed [30]. To 1 mL of the lipid fraction of the extracts a methylation with 5 mL of methanol:sulphuric acid:toluene 2:1:1 (v:v:v) for at least 12 h in a bath at 50 °C and 160 rpm was done. The recovered FA methyl esters (FAME) were treated with 3 mL of diethyl ether by shaking in vortex after water addition for phase separation. The water was eliminated by passing the upper phase through a micro-column of sodium sulfate anhydrous. The lipid fraction was quantified using chromatograph-GC 1000 with a flame ionization detector (FID) (GC-FID; DANI1000, Contone, Switzerland) set at 260 °C and a Zebron-Kame column (30 m × 0.25 mm ID × 0.20 μm df, Phenomenex, Lisbon, Portugal) as well as a split/splitless injector set at 250 °C with a split ratio of 1:50. The relative retention times of each FAME peak were used to identify and quantify FA samples by comparison with those of standards (reference standard mixture 47885-U, Sigma, St. Louis, MO, USA). The results were expressed in relative percentage of each FA and were analysed using the Software Clarity DataApex 4.0 Software (Prague, Czech Republic).

2.10. Statistical analysis

The results were statistically analyzed using the GraphPad Prism 8.0.2 software for analysis of variance (One-way and two-way ANOVA) followed by Tukey or Sidak multiple comparison test. Differences were considered significant if $p < 0.05$. For results regarding the composition of fatty acids present in the extracts, data based on the descriptive statistics with means and standard deviations were presented.

3. Results & discussion

In the present work, different extraction methods of the lipid fraction obtained from BSF biomass were compared in terms of yields, lipid composition, antioxidant capacity, and general toxicity.

From Figure 1, it is possible to observe the yields obtained from each extraction method, carried out in two cycles.

It is known that organic solvents, according to their polarity, can extract different classes of compounds [20, 31], which can justify the better performance results for *n*-hexane and acetone when compared to different extraction techniques using water. Extractions with *n*-hexane obtained significantly better yields when compared with those obtained with acetone in the first cycle ($p = 0.0003$), but this difference was not significant in the second cycle, i.e., when this solvent was used with ultrasounds extraction. Among the aqueous extraction methods,

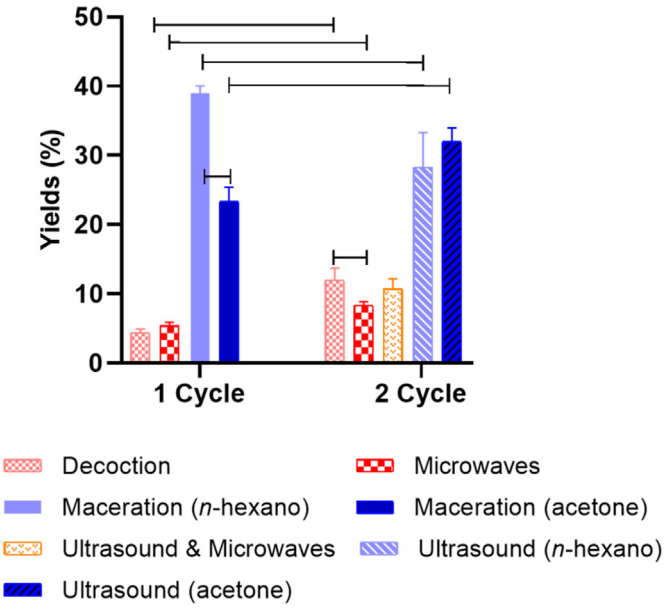

Figure 1. Yields obtained from different extraction methods. The bars represent the significant *p*-values comparing first and second cycle yields: decoction - 0.0017; microwave - 0.0009; maceration x ultrasound with *n*-hexane - 0.0215; maceration x ultrasound with acetone - 0.0056.

differences were only observed between decoction and microwave extractions in the second cycle ($p = 0.0157$). The combination of ultrasound followed by microwaves did not significantly improve the yields when compared to those in which water was used as a solvent. Grinding the larvae before the aqueous extractions processes in the second cycle resulted in a significant increase in yields. We observed that, when grinding the larvae, the rigid barrier of the chitin-rich exoskeleton is broken and can facilitate the release of lipid compounds. Despite the relatively low yields of aqueous extraction compared to those achieved using organic solvents, aqueous methods have advantages in that they do

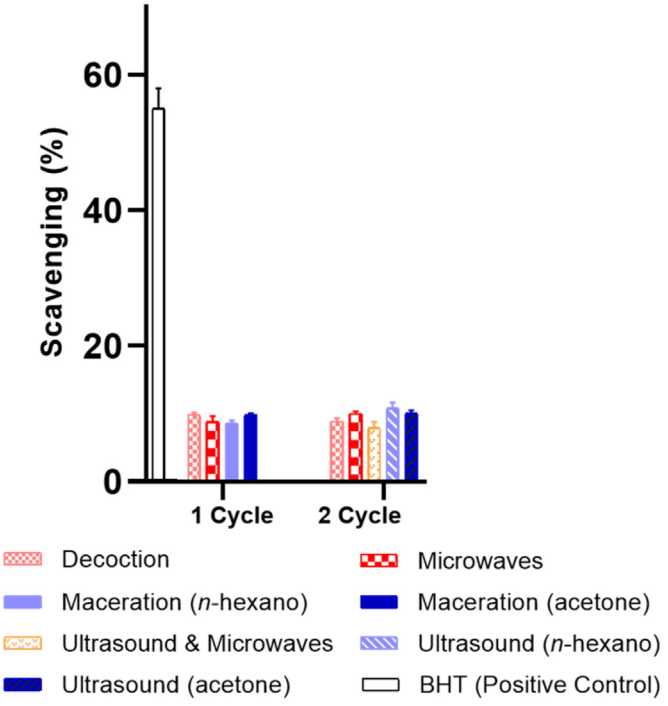

Figure 2. Graph of the antioxidant activity from the percentages of absorbance obtained by the extracts of each method.

Table 1. Fatty acids composition (%) of the extracts from BSF larvae lipid fraction (mean ± SD).

Fatty acids	First cycle				Second cycle				
	Decoction	Microwaves	Maceration with *n*-hexane	Maceration with acetone	Decoction	Microwaves	Ultrasound & microwaves	Ultrasound with *n*-hexane	Ultrasound with acetone
Capric acid (C10:0)	1.45 ± 0.01f,g	1.45 ± 0.04f,g	1.49 ± 0.04f	1.39 ± 0.03g	2.3 ± 0.1a	2.2 ± 0.1b	1.97 ± 0.07c	1.66 ± 0.01e	1.78 ± 0.02d
Lauric acid (C12:0)	42.0 ± 0.2d	41 ± 1e	38.7 ± 0.8f	37 ± 1g	59.7 ± 0.8b	62.2 ± 0.9a	59.3 ± 0.8b	54.4 ± 0.2c	54.3 ± 0.1c
Myristic acid (C14:0)	8.70 ± 0.04d	8.5 ± 0.2e	7.59 ± 0.08f	7.1 ± 0.2g	9.9 ± 0.1c	11.4 ± 0.1a	10.1 ± 0.1b	10.0 ± 0.1c	9.89 ± 0.02c
Myristoleic acid (C14:1)	0.216 ± 0.001b	0.199 ± 0.002c	0.21 ± 0.01b	0.23 ± 0.01a	0.053 ± 0.001g	0.057 ± 0.004g	0.063 ± 0.003f	0.075 ± 0.006e	0.102 ± 0.006d
Penta-decanoic acid (C15:0)	0.22 ± 0.01a	0.22 ± 0.01a	0.22 ± 0.01a	0.21 ± 0.01b	0.192 ± 0.004c	0.218 ± 0.004a,b	0.19 ± 0.01c	0.176 ± 0.006d	0.169 ± 0.005d
Palmitic acid (C16:0)	14.21 ± 0.05a,b	14.5 ± 0.1a	14.25 ± 0.05a	13.77 ± 0.03b	11.7 ± 0.5e	12.1 ± 0.8d	11.9 ± 0.9d,e	12.7 ± 0.7c	12.11 ± 0.01d,e
Palmitoleic acid (C16:1)	3.32 ± 0.06c	3.21 ± 0.01d	3.49 ± 0.07b	3.77 ± 0.02a	1.49 ± 0.01g	0.82 ± 0.04i	1.6 ± 0.1h	2.55 ± 0.06f	2.65 ± 0.09e
Hepta-decanoic acid (C17:0)	0.334 ± 0.003b	0.346 ± 0.005a	0.35 ± 0.01a	0.33 ± 0.01c	0.260 ± 0.004d	0.256 ± 0.004d	0.236 ± 0.006e	0.163 ± 0.003g	0.20 ± 0.01f
Stearic acid (C18:0)	2.25 ± 0.03c	2.4 ± 0.1b	2.51 ± 0.05a	2.5 ± 0.1a	1.47 ± 0.05e	1.65 ± 0.01d	1.62 ± 0.06d	1.52 ± 0.07e	1.51 ± 0.01e
Oleic acid (C18:1n9c)	11.2 ± 0.1d	11.6 ± 0.5c	13.1 ± 0.2b	13.7 ± 0.6a	5.7 ± 0.3f	4.26 ± 0.08g	5.64 ± 0.02f	8.2 ± 0.2e	8.35 ± 0.03e
Linoleic acid (C18:2n6c)	14.58 ± 0.09d	15.1 ± 0.6c	16.4 ± 0.4b	17.7 ± 0.7a	6.62 ± 0.02g	4.51 ± 0.01h	6.7 ± 0.4g	8.0 ± 0.2f	8.3 ± 0.1e
Alpha-linolenic acid (C18:3n3)	1.55 ± 0.01d	1.63 ± 0.06c	1.73 ± 0.04b	1.95 ± 0.04a	0.61 ± 0.01g	0.32 ± 0.01h	0.64 ± 0.01e,f	0.62 ± 0.03f,g	0.66 ± 0.01e
SFA	69.17 ± 0.08e	68 ± 1f	65.1 ± 0.8g	63 ± 1h	85.6 ± 0.3b	90.1 ± 0.1a	85.4 ± 0.2b	80.7 ± 0.4c	80.0 ± 0.1d
MUFA	14.7 ± 0.2d	15.0 ± 0.5c	16.8 ± 0.3b	17.7 ± 0.6a	7.2 ± 0.3f	5.1 ± 0.1g	7.3 ± 0.2f	10.8 ± 0.2e	11.0 ± 0.1e
PUFA	16.12 ± 0.09d	16.8 ± 0.6c	18.1 ± 0.4b	19.6 ± 0.8a	7.2 ± 0.1g	4.8 ± 0.1h	7.3 ± 0.4g	8.5 ± 0.2f	9.0 ± 0.1e

SFA - saturated fatty acids; MUFA - monounsaturated fatty acids; PUFA - polyunsaturated fatty acids. Different letters in the same line mean significant differences ($p<0.05$).

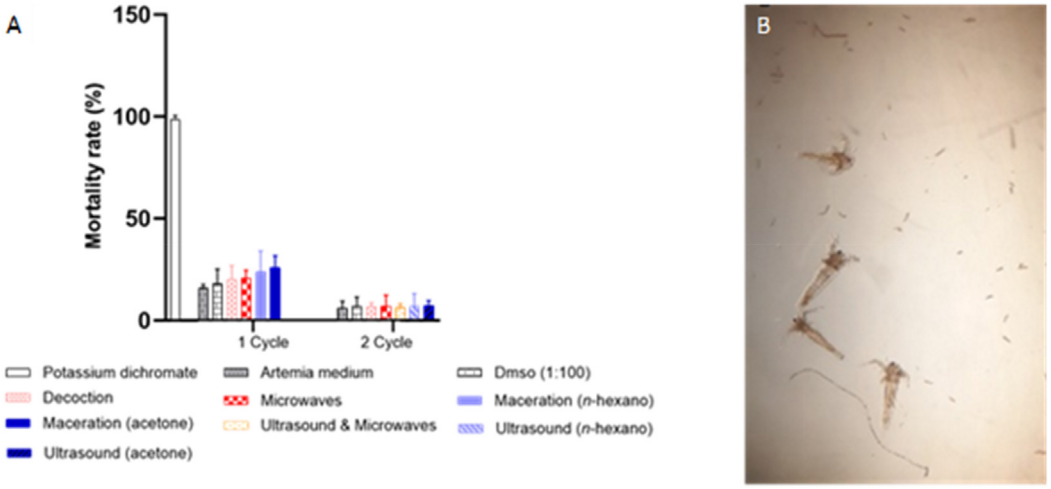

Figure 3. General Toxicity bioassay using *A. salina*. A) Graph with the mortality rate of nauplii; B) Alive *A. salina* nauplii.

not involve excessive solvent consumption, require lower energy, and are also easily applicable on a larger scale and, thus, can be considered more sustainable.

3.1. Antioxidant activity (DPPH)

The assessment of the antioxidant activity of the extracts by the reduction of free radicals and consequent discoloration reaction of DPPH is shown in Figure 2. From the quantitative analyses by spectrophotometry by measuring the absorbance from reactivity of DPPH with each extract, it is noted that the extracts studied exhibited low antioxidant activity at a concentration of 0.1 mg mL^{-1}. No significant differences were observed between the results of the first and second cycles. The low percentage of antioxidant activity may be due to the prior dilution of the extract samples and consequent reduction in the concentration of fatty acids with these properties in the lipid composition of the extracts (see Table 1). Further studies may be needed in the future to assess the antioxidant activity of the extracts.

3.2. General toxicity bioassay (Artemia salina model)

The mortality percentages after 24 h of exposure of the nauplii to the components of the extracts are shown in Figure 3. After the total count of nauplii killed in each well, it was possible to establish a rate with those quantified initially in each well. As can be seen in the figure, the extracts were not significantly lethal at the applied concentration, especially when compared to the potassium dichromate positive control. That is, the tested extracts maintained the *A. salina* viability well above 50%, being, thus, considered non-toxic [29]. It should be noted that the mortality rates of the extracts were not relevant because they were very similar to the negative controls, those obtained by the saline medium (*A. salina* growth medium) and those from the solvent in which the extracts were dissolved.

3.3. Profile and fatty acids composition of the extracts

The results of the lipid composition of the extracts obtained using different extraction processes in the first and second cycles are presented in Table 1. The FA profiles are similar between extractions and corroborate those described in the literature [10, 11, 12, 32, 33, 34, 35]. The lauric acid content (C12: 0) is prevalent in all extractions in relation to the other FA, followed by the content of palmitic (C16: 0) and linoleic (C18: 2) acids. However, it should be noted that different extraction methods provided slightly different concentrations of FA classes. Higher concentrations of lauric acid were achieved through aqueous extractions, providing 41%–62% in the extract. Despite having low yields when compared to those obtained by organic solvents (4% from decoction against 39% using hexane), the aqueous extractions, specifically by microwaves in the second cycle (around 12% yield), proved satisfactory in obtaining higher concentrations of lauric acid (62%), as showed in Figure 4. Thus, according to the different extraction methods applied, it was possible to extract from the BSF larvae varying amounts of lauric acid (C12:0) (37–62%), palmitic acid (C16:0) (11–14%), linoleic acid (C18:2n6) (4–17%) and oleic acid (C18:1n-9) (4–13%). Our results differ slightly from those achieved by Ewald *et al.* [35] who reported that the largest amounts of FA extracted from the larvae were lauric (52%), palmitic (12–22%), and oleic (10–25%). The work of Marusich *et al.* [36] reports 22% of oleic (C18:1n9), 20% of palmitic (C16:0), and only 18% of lauric acid (C12:0) after using extraction techniques with acidified water-methanol. Comparing yields of the solvents with similar polarity, satisfactory results were achieved by Wong *et al.* [37], using petroleum ether in a 24-hour maceration process, resulting in higher yields of fatty acids. However, when combining n-hexane with ultrasound in our studies, an approximate percentage of lauric acid was reached, but in less extraction time. The differences in the percentages of extracted FA reported in different studies can be attributable to the methods of extraction applied and the type of solvent used. However, it is important to highlight that the lipid composition profile will also be significantly dependent on the type of substrate used for rearing the larvae [2, 34, 38, 39].

The omega-6 fatty acids (ω-6 PUFAs), mainly Linoleic acid (C18: 2n6c) (LA), were also identified in the lipid composition of BSF larvae biomass, with a variation from 14.58% to 17.7% in the first cycle and with 4.5%–8.3% in the second. In the omega-3 group, namely α-Linolenic acid (C18: 3n3) (ALA), values of 1.55%–1.95% and 0.32%–0.66% were reached, in the first and second cycle, respectively. Hoc *et al.* [10] obtained a higher percentage of omega-6 and 3 when introducing to the diet of the larvae sources rich in polyunsaturated fatty acids from agricultural by-products (rape and flax cakes), having as one of the objectives the reduction of the rates of SFAs in the pre-pupae of the BSF. These results demonstrate that the BSF larvae nutritional composition is related to their diet and can, therefore, be manipulated.

The extractions using organic solvents provided higher concentrations of PUFA in both cycles. Grinding the larvae before the extraction process in the second cycle resulted in increased yields for aqueous extractions, however, a reduction in the percentage of PUFA was observed. When the larvae are intact during decoction and microwaves, the presence of a rigid exoskeleton rich in chitin, can act as a protective barrier against heating and prevent the possible degradation/reduction of PUFA.

The role of essential FA in the human epidermis is widely acknowledged [14]. Palmitic acid and its derivatives are emollients and linoleic

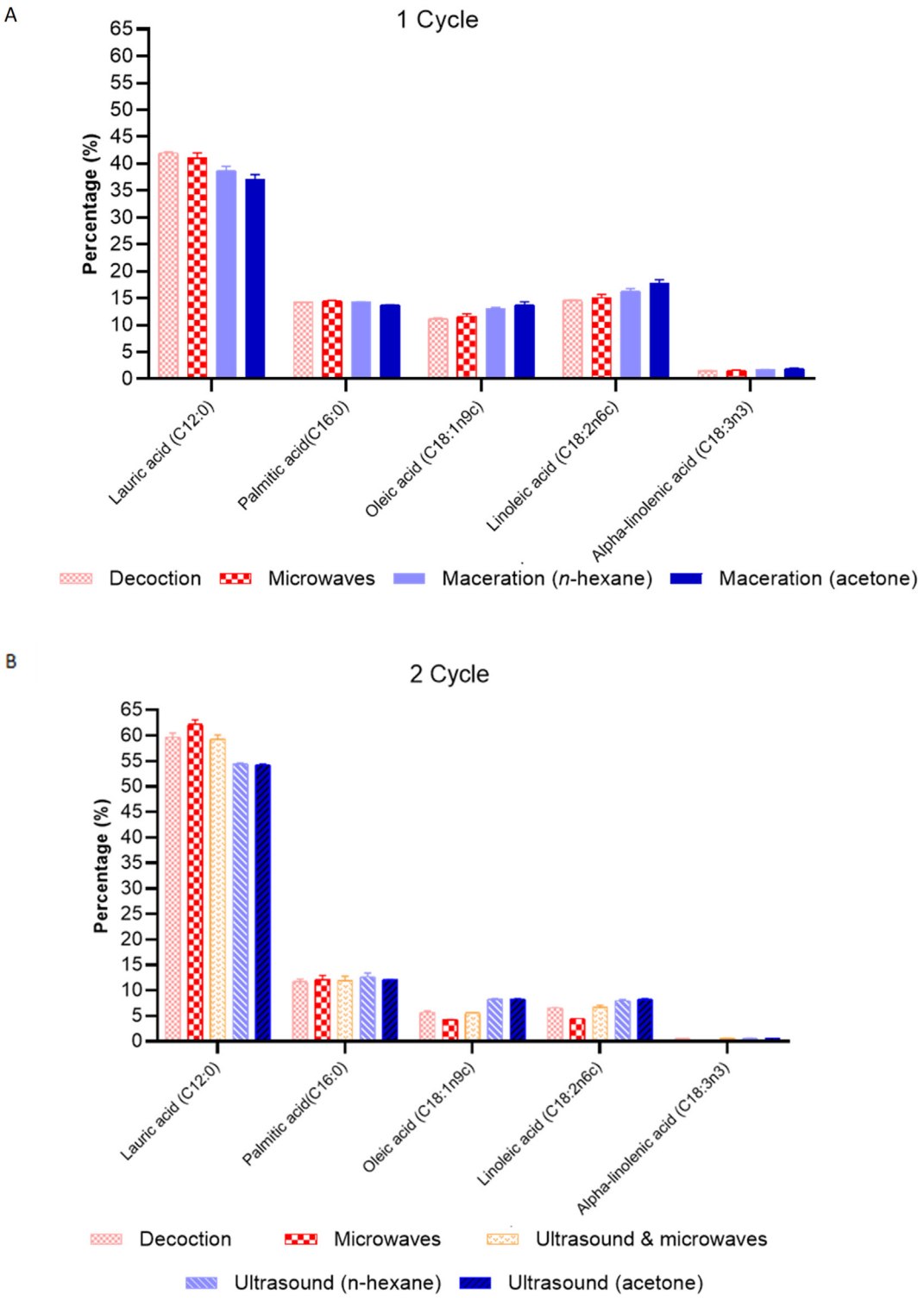

Figure 4. The main fatty acids composition in the BSF larvae lipid extracts. A) The first cycle of extraction; B) The second cycle of extraction.

acid contributes to water impermeability of the *stratum corneum* since it is a constituent of acylglycosyl ceramides [16]. Myristic acid is capable of repairing epidermal barrier properties, as well as act as penetration enhancer [15]. Oleic acid regulates the epidermal lipid metabolism, thus impacting *stratum corneum* barrier and hydric content [15]. Omega-3 (ω-3) and omega-6 (ω-6) FA have been reported to reduce inflammation and can have a significant role in formulations aiming at inflammatory skin diseases such as atopic dermatitis or psoriasis [40, 41].

FA extracted from the BSF larvae can also have a role as antimicrobial assets. There is a growing interest in new actives with antimicrobial effects in view of the problem of resistance to antibiotics [36, 42] and consumer distrust in preservatives used in cosmetic formulations [17]. As mentioned previously, lauric acid has been shown to have antimicrobial activity [13], and it can be converted into monolaurin, which is known to be antiviral, antibacterial, and antiprotozoal [11].

As mentioned, FA are important constituents of the skin lipid barrier and the use of these substances as ingredients in formulations has increased due to their functional effects (biological activities) and their natural biocompatibility [14]. From the results obtained in this work, it is suggested that the lipid content present in the biomass extracts of BSF larvae could be applied in topical and cosmetic formulations as a blend of FA. The lauric acid and other FA present in the BSF larvae oil can be applied either isolated or a in lipid structural complex and can be foreseen to be used as skin emollients, as structures for the encapsulation of different active substances, or to perform other roles in formulations [2, 11, 43].

4. Conclusions

The results obtained in the present study show that the choice of the type of pre-treatment given to the larvae biomass and the applied extraction technique significantly affect the compounds that are extracted, namely the fatty acids profile. In the preliminary screening of its bioactivity and safety, the lipid extracts had a limited antioxidant performance, however, none of our extracts proved to be toxic in the concentrations applied in the tests with *Artemia salina*.

This study assessed the potential application of BSF larvae extracts obtained from different extraction methods. Through this study, it was possible to obtain a mixture of fatty acids, highly rich in lauric acid, which is very promising for pharmaceutical and cosmetic application, and with good prospects in biocompatibility with skin. In addition, it was possible to obtain an insect oil containing high concentrations of lauric acid by applying an aqueous extraction method that is relatively economical, simple, sustainable, with potential development for industrial application.

Declarations

Author contribution statement

Cíntia Almeida, Ângela Fernandes: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Daniel Murta, Rui Nunes, André Rolim Baby: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Lilian Barros: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Patricia Rijo, Catarina Rosado: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This work was supported by national funds through FCT - Foundation for Science and Technology, I.P., under the UIDB/04567/2020 and UIDP/04567/2020 projects attributed to CBIOS, and UIDB/00690/2020 to CIMO and by the PhD grant attributed to C.A. (UI/BD/151423/2021). National funding by FCT, P.I., through the institutional scientific employment program-contract for the contracts of A. Fernandes and L. Barros. Additionally, the authors would like to acknowledge funding of the projects ENTOVALOR (POCI-01-0247-FEDER294 017675/ 2016–2019) and NETA (POCI-01-0247-046959).

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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