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Food Chemistry

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https://doi.org/10.1016/j.foodchem.2021.129077

Food Chemistry 349 (2021) 129077

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Lipidome of cricket species used as food

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ARTICLE INFO

Keywords:
Novel food
Lipidomics
Entomophagy
Insect oil
Insects as food

ABSTRACT

The variation in lipidome of house cricket, banded cricket, Jamaican field cricket and two-spotted cricket was studied using high-throughput screening techniques for fingerprinting (MALDI TOF MS, GC–MS and LC MS-MS) and well-stablished chromatographic techniques for quantification (HPLC-ELSD, GC- FID). Although the four cricket species were reared in identical conditions, two-spotted & banded crickets had a lipid content 1.5 fold higher than house cricket. The lipids were high in UFA (>63%) and unsaturated TAG (>98%) making them liquid at room temperature, thus an oil. Cholesterol and several phytosterols were profiled finding high cholesterol concentration which is a point of concern. Eight phospholipid types (211 species) were identified with no major differences among cricket species. Using high-throughput screening techniques we demonstrate the complexity of cricket lipidome. Information on the lipidome of these crickets with high commercial value is important to estimate its nutritional value and their potential food applications.

1. Introduction

The global market for edible insects has steadily increased in the past years and is predicted to grow by 24.4% annually reaching a turnover of USD7.96 billion by 2030 (MeticulousResearch, 2019). From all insect species produced for food, cricket species have the largest market share (MeticulousResearch, 2019). Indeed, about 15% of the companies producing insects in Europe focus on the house cricket (*Acheta domesticus*) according to the International Platform of Insects for Food and Feed (IPIFF) (Derrien, 2017). Besides the house cricket, also banded crickets (*Gryllodes sigillatus*), Jamaican field crickets (*Gryllus assimilis*), and two-spotted crickets (*Gryllus bimaculatus*) are commercially reared for human consumption around the world (Halloran, Hanboonsong, Roos, & Bruun, 2017).

Whole crickets and cricket meals are mainly appreciated in the edible insect market for their protein content, leaving aside the nutritional value of their lipid fraction which includes antioxidants and ω-3 fatty acids (FAs) (del Hierro, Gutierrez-Docio, Otero, Reglero, & Martin, 2020; Tzompa-Sosa, Yi, van Valenberg, van Boekel, & Lakemond, 2014). However, these lipid fractions can also be valorized as food ingredients, increasing the environmental and economic sustainability of insect production. Utilization of insect oils and fats as food ingredients requires deep understanding of their chemical composition, as this determines its nutritional quality and its technological and functional properties in food products systems. Detailed information on the lipid chemical composition of cricket species is lacking in the scientific literature.

The food industry valorizes lipids in different ways. For instance, phospholipids are the main component of lecithin (about 60%), which is widely used as emulsifier, anti-dusting agent, viscosity modifiers, as well as wetting, release or separating agent (Szuhaj, 2003). Triacylglycerols are the main lipid of edible fats and oils. Hence, the chemical composition and physical properties of these triacylglycerols will determine the functionality of fats and oils in fat rich products (Indelicato et al., 2017; Sato & Ueno, 2014). Furthermore, sterols, specifically phytosterols, are considered a functional ingredient improving the lipoprotein profile of consumers by lowering levels of LDL cholesterol and reducing intestinal cholesterol absorption (Grille, Zaslawski, Thiele, Plat, & Warnecke, 2010). Studying the lipid profile of commercially reared

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https://doi.org/10.1016/j.foodchem.2021.129077
Received 15 October 2020; Received in revised form 23 December 2020; Accepted 7 January 2021
Available online 19 January 2021

cricket species helps to understand their nutritional value, their potential food applications and opens ways to valorize them. However, different cricket species might well have the ability to synthesize different lipid species or to synthesize them in different amounts.

Hence, the aim of this study was to determine the variation in lipid profiles of four cricket species by identifying the compounds present in both the polar and non-polar fraction. We hypothesized that, lipid metabolism differs among species of the Gryllidae family, this will be reflected on the fat being stored in its body (lipid content) and on its lipidome. To test this hypothesis, we reared four cricket species under the same conditions and profiled its lipid classes. We profiled FA, phospholipids, triacylglycerols, and sterols using high-throughput screening techniques for fingerprinting and well-stablished chromatographic techniques for quantification. This information was contrasted with other edible oils and potential food applications for cricket oils were defined.

2. Materials and methods

2.1. Cricket species and rearing

Four cricket species with industrial relevance were selected; house crickets, banded crickets, Jamaican field crickets and two-spotted crickets. All crickets were reared in a climate-controlled room (28 °C, 50% relative humidity) under a light regime of 12:12 (Light:Darkness). Newly eclosed house crickets were taken from colonies maintained at the Laboratory of Entomology, of Wageningen University & Research, Wageningen, the Netherlands. Eggs of banded crickets and Jamaican field crickets were provided by Kreca V.O.F. (Ermelo, The Netherlands) and were hatched at the laboratory of Entomology, Wageningen University, Wageningen, the Netherlands. Adult Jamaican field crickets were provided by Starfood (Barneveld, the Netherlands) and allowed to mate and oviposit in the aforementioned climate chamber. General cricket rearing procedures were described in detail by Veenenbos and Oonincx (2017). In short, 100 newly eclosed crickets were setup in a plastic container (Faunarium pt2265, Hagen, Holm, Germany), containing two halves of an egg tray, and provided with water and chicken feed diet *ad libitum*. This feed was used since it is considered a basal diet for crickets and is easily accessible (Oonincx, Laurent, Veenenbos, & Loon, 2019). When the first five crickets in a container reached adulthood, as indicated by the development of full-size wings, the container was placed at −18 °C for 30 minutes. Then, all crickets from that container were put in a plastic bag and kept frozen at −18 °C until further processing. In the next step, crickets from two containers were combined and the gender of the crickets was determined. As crickets have a 1:1 sex ratio a sample consisting of 50% males and 50% females was considered most representative for the chemical composition in large scale production. Hence, this sampling can be considered representative of commercially produced crickets. This sampling resulted in 3 gender-corrected samples per cricket species, each corrected sample was considered a biological replicate. The dry matter content of these samples was determined by freeze drying (Model GRI 20–85 MP 1996, GR Instruments B.V., the Netherlands). Then, the samples were ground to a fine powder using a Waring blender (model 34BL99, Dynamics Corp. of America, New Hartford, CT, USA) and stored at −20 °C until lipid extraction.

2.2. Lipid extraction

Total lipid content was determined via Folch extraction. Five grams of the freeze-dried cricket powder was placed in a glass flask mixed with 85 mL dichloromethane/methanol (2:1) solution and shaken for 2 h on a rotary shaker (Edmund Bühler Gmbh SM-30, Hechingen, Germany). After adding 21.2 mL of demineralized water, the mixture was centrifuged at 2000*g* for 20 min at room temperature, resulting in two layered fractions. The upper aqueous layer containing non-lipid compounds was removed from the flask with a glass pipette. The lower layer, composed of a mixture of organic solvent (86:14:1, dichloromethane/methanol/water) and insect tissue, was left standing for 12 h to aid lipid extraction. Subsequently, this fraction with solubilized lipids was filtered using a paper filter and transferred to a round-bottom glass flask. The flasks and filters were flushed twice with dichloromethane to avoid losses. Next, the mixture was dried using a rotary evaporator flushed with nitrogen at 400 mbar in a water bath at 40 °C. The extracted lipids were weighed and the lipid concentration calculated. These were stored under nitrogen atmosphere at −20 °C until further analysis. Three technical replicates of the lipid extraction were performed per biological replicate (3 technical replicates × 3 biological replicates). The lipid fraction from the feed (dry feed and carrots) was also extracted in triplicate (3 biological replicates × 3 technical replicates). Afterwards, the fat from each technical replicate was pooled within each biological replicate and stored at −20 °C until further analysis. This fat extract was used for all subsequent lipid analysis.

2.3. FA composition

Fatty acids were analyzed as fatty acid methyl esters prepared by using boron trifluoride in methanol as described by Metcalfe, Schmitz, and Pelka (1966). Fatty acid methyl esters (0.5 μl) were then analyzed by gas chromatography (Agilent 6890N GC; Agilent Technologies, USA) with an FID detector using a CP-Wax 58 FFAP CB column (25 m × 0.25 m, i.d. 0.2 mm). Identification was done using a standard fatty acid methyl esters mixture. Each of the three biological replicates of the cricket species and the feed was analyzed in duplo. The results are shown in g/100 g of total fatty acids (TFA).

2.4. Phospholipid identification by liquid chromatography- mass spectrometry (LC – MS)

For the determination of PL composition, the dry lipids extracted were dissolved in 1000 μl chloroform / methanol (1/1; v/v). Then, 1–10 μl was injected directly onto a hydrophilic interaction liquid chromatography column (2.6 μm HILIC 100 Å, 50 × 4.6 mm, Phenomenex, Torrance, CA) and eluted with a gradient from acetonitrile/Acetone (9:1, v/v) to acetonitrile/H_2O (7:3, v/v) with 50 mM ammonium formate, and both with 0.1% formic acid as described previously (Jeucken & Brouwers, 2019). The column outlet of the chromatograph was either connected to a heated electrospray ionization source of a LTQ-XL linear ion trap mass spectrometer or a Fusion mass spectrometer (both from Thermo Fisher Scientific, Waltham, MA), collecting full scan- and data dependent MS2 spectra in the negative ionization mode. One run was performed per biological replicate.

2.5. Profiling of non-polar lipids by MALDI-TOF MS

First, the non-polar fraction of the extracted cricket lipids was isolated from the total cricket lipids (100 mg of oil) as described by Kaluzny, Duncan, Merritt, and Epps (1985) and dissolved in a minimal amount of chloroform (≤ 200 μl). Isolation was performed using aminopropyl solid phase extraction columns (Extract-Clean SPE NH2, 1000 mg/8mL, Grace Discovery Sciences) and a Vac Elute apparatus under vacuum. The recovered fraction was dried with nitrogen flow and used for TAG profiling by HPLC - ELSD and MALDI-TOF MS. To confirm the absence of polar lipids in the recovered fraction, identification of lipid classes by thin layer chromatography was performed as described by Tzompa-Sosa et al. (2014). Thin layer chromatography showed spots in the region corresponding to TAG, non-polar sterols/waxes and DAG (Supplements Fig. S1). Therefore, non-polar lipids (TAG, sterol and waxes) and DAG were selected as categories for mass identification using the Lipid Maps tool (Fahy, Sud, Cotter, & Subramaniam, 2007).

The non-polar fraction of the cricket lipids was then profiling by MALDI-TOF MS (Ultraflextreme, Bruker, Germany), and the data was

processed as described by Tzompa-Sosa, Meurs, and van Valenberg (2018). Each biological replicate was analyzed in septuplicate (3 biological replicates × 7 technical replicates). The mean of a biological replicate was the average of those seven replicates. The results reported are the mean of three biological replicates. Each detected mass was assigned to a lipid specie using a computationally-generated database of 'bulk' lipid species (COMP_DB) mass spectrometry peak prediction available online at LIPID MAPS®(Fahy et al., 2007). The categories searched for were triacylglycerols, diacylglycerols, wax esters and cholesteryl esters. To avoid misidentification, further lipid identification was done taking into account the FA composition of each sample.

2.6. TAG profile and quantification by HPLC - ELSD

The non-polar fraction of cricket lipids separated by solid phase extraction (Section 2.5) was used to profiled TAG as described by Rombaut, De Clercq, Foubert, and Dewettinck (2009). In short, this fraction was dissolved in a 30/70 v/v mixture of dichloromethane/acetonitrile in a vial at a concentration level of 5 mg/mL. Then, 25 μl of this solution was injected and gradiently separated by an RP-HPLC device (Thermo Finnigan Surveyor, Thermo Electron Corporation, Brussels, Belgium) using a C18 column (Alltima HP C18 HL, 150 × 3.0 mm, 3 μm particle diameter, Grace Alltech, Lokeren, Belgium) connected to an Evaporative Laser light Scattering Detector (Alltech ELSD 2000, Grace, Lokeren, Belgium). Each of the three biological replicates was analyzed in duplo.

For the identification of the eluted peaks, TAG internal standards were selected and spiked in the sample vial. These internal standards were set as anchor points at the beginning, end and within the TAG elution range. These standards were tridecanoin (c13:0-c13:0-c13:0) at the beginning of the TAG range. Triheptadecanoin (c17:c17:0-c17:0) at the end. And in between, trihexadecanoin (c16:0-c16:0-c16:0) and triolein (c18:1-c18:1-c18:1). All standsrds were purchased from Nu-Chek Prep (USA). Also 1,2-palmitin-3-olein (c16:0-c16:0-c18:0 (PPO)) from Larodan AB (Sweden) was used. Besides these standards, a cocoa butter standard (IRMM-801) was used in which 21 TAGs were present. Cocoa butter standard was used since it covered part of the range of the TAG in cricket lipids, it is a well characterized fat and helped to confirm TAG identification in these cricket oils.

2.7. Sterol quantification and identification by GC-FID and GC–MS

Total cricket lipid extracts from Folch extraction (Section 2.2) were used for sterol quantification and identification. After saponification of the lipid extract, the sterols in the unsaponifiable fraction were determined gas chromatographically via true silylation in accordance with DGF Method F-III 1 (DGF, 2019). Quantification was done via GC-FID (Agilent 6890; Agilent Technologies, USA) with a highly inert, nonpolar, 100% dimethylpolysiloxane phase column (Agilent J&W VF-1 ms, 30 m, 0.25 mm, 0.25 μm). The standards used were cholesterol (Acros organics; 95%), stigmasterol (Sigma Aldrich; 95%), and a mixture of β-sitosterol (≥40%) and campesterol (40%) (Sigma Aldrich). Untargeted profiling of sterols was performed by GC–MS (Agilent 7890/5975; Agilent Technologies, USA) using a highly inert, nonpolar, 100% dimethylpolysiloxane phase column (Agilent J& W VF-1 ms GC Column, 30 m, 0.25 mm, 0.25 μm). NIST-library was used for lipid identification. Two biological replicates per species were analyzed in duplo.

2.8. Statistical analysis

The relative concentrations of the FAs (GC-FID), TAG (by HPLC-ELSD) and phospholipid abundance (LC–MS) were compared among cricket species. The mean values shown are based on biological replicates, while technical replicates were used to estimate the reproducibility of the assay. Assumptions of normality and equality of variance were tested prior to ANOVA analysis using Shapiro-Wilk's test and Levene's test, respectively. Where assumptions were fulfilled, ANOVA at a 5% significance level with a post-hoc Tukey's test was used to investigate significant differences among samples. However, when equality of variances was not met, Games Howell post-hoc test was performed. Principal component analysis (PCA) was carried out to determine groupings between cricket species and to investigate the relationship with phospholipid relative abundance. A 5% significance level was applied for all tests. All statistical analysis were performed with IBM SPSS statistics software (Version 25, Armonk, NY).

3. Results and discussion

3.1. Fat content and FA composition

House crickets had the lowest fat content on a dry matter basis (22%, $p < 0.05$), followed by Jamaican field crickets (28%, $p < 0.05$), whereas the fat content of two-spotted crickets (33%) and banded crickets (32%) were the highest ($p < 0.05$) (Table 1). As rearing conditions, including the feed, were identical this could indicate differences in nutritional physiology and metabolism between the four species. The fat content (as % of dry matter) from our crickets was fairly high compared to previously reported data of reared crickets which is between 13 and 23% for house crickets (Kulma et al., 2019; Oonincx, van Broekhoven, van Huis, & van Loon, 2015), 18–20% for banded crickets (Józefiak et al., 2018; Zielińska, Baraniak, Karaś, Rybczyńska, & Jakubczyk, 2015), 12–23% for Jamaican field crickets (Mlček et al., 2018; Soares Araújo, dos Santos Benfica, Ferraz, & Moreira Santos, 2019) and 12–27% in dry matter basis for two-spotted field crickets (Ghosh, Lee, Jung, & Meyer-Rochow, 2017; Hoffmann, 1973). Feed composition was the main cause of variation in fat content among studies. The relatively high fat content in our study was likely due to a high feed quality and low rearing densities during cricket rearing.

The FA composition (Table 1) of the crickets was approximately equally divided between PUFAs (35.3–37% TFA), SFA (32.7–36.5% TFA) and MUFA (25.9–31.2% TFA). The main FA in the cricket oils were C16:0, C18:1n-9 and C18:2n-6. Accounting for > 83% TFA. Our results are within the ranges for these cricket species reported in literature (Mlček et al., 2018; Oonincx et al., 2015; Soares Araújo et al., 2019; Tzompa-Sosa et al., 2014). These profiles largely reflected the FA profiles of the provided feed. In these previous studies, the concentration range of C18 FA varied more than that of C16:0. This could suggest that C16:0 is more strongly regulated than C18 FAs, or that C16:0 is synthesized *de novo* or that C18 FA concentrations vary more in the feed used in the different studies. Small but significant ($p < 0.05$) variability within the cricket species was observed in the concentration of C16:0, C18:1n-9 and C18:2n-6 FA. The most striking difference in these FA was the increase in C18:1n-9 in two-spotted cricket which was on average 4.38% of TFA higher than in the other three species.

In the cricket feed (chicken feed and carrot) linoleic acid (C18:2n-6) was the most abundant FA (>44.5% TFA) (Table 1), surpassing the concentration found in the crickets (34–35% TFA). According to Stanley-Samuelson, Loher, and Blomquist (1986), in testicular tissue of Australian field cricket, a small portion of C18:2n-6 is transformed into C20:3n-3 and the rest remained untransformed. In wax moth, C18:2n-6 elongates and desaturates to 20:4n-6 (Stanley-Samuelson, Jurenka, Loher, & Blomquist, 1987). In our study, is likely that C18:2n-6 remained untransformed and the excess was either used in other metabolic pathways or converted to FAs other than C20:3n-3 or 20:4n-6 since these latest FA were not identified in any cricket species. Elongation and desaturation seems unlikely in our study since the concentration of C20 FA is minimal. To the best of our knowledge, this pathways are yet to be described but it is important to undelved the faith of C18:2n-6 since this FA is highly present in reared insect diets. The second most abundant FA in crickets was C18:1n-9. One part could have come from feed and another part could have been *de novo* synthesized from C2:0, as described by Blomquist, Borgeson, and Vundla (1991). C18:0 could have

Table 1
Lipid content dry matter and fatty acid composition of four cricket species and its feed (chicken meal and carrot) (mean ± S.D.. N = 3)[1,2].

	Feed						Cricket species																	
	Dry feed			Carrot			House cricket *(Acheta domesticus)*				Banded cricket *(Gryllodes sigillatus)*				Two-spotted cricket (*Gryllus bimaculatus*)				Jamaican field cricket (*Gryllus assimilis*)			
Lipid content (dry basis)	4.65	±	0.05	1.82	±	0.29	22.36	±	0.28	c	32.41	±	2.67	a	32.95	±	0.83	a	27.80	±	0.46	b
Lipid content (wet basis)	4.19	±	0.05	0.20	±	0.04	5.96	±	0.39	c	10.55	±	0.85	a	10.03	±	0.30	a	8.03	±	0.04	b
Dry matter	90.16	±	0.16	10.92	±	0.51	27.16	±	1.33	d	31.9	±	1.33	c	30.43	±	0.13	a	28.95	±	0.47	b
Fatty acids																						
C14:0	0.51	±	0.05				0.69		0.07	a.b	0.80		0.01	c	0.57		0.02	a	0.82		0.03	b.c
C15:0				0.81	±	0.04													0.05	±	0.09	
C16:0	15.86	±	0.19	18.63	±	0.29	26.14	±	1.57	b	24.61	±	0.26	a.b	24.09	±	0.14	a	24.50	±	0.51	a
C16:1n-9							0.43	±	0.02	c	0.39	±	0.03	a	0.34	±	0.01	a	0.55	±	0.01	b
C16:1n-7	0.66	±	0.03				0.84	±	0.11	a	1.40	±	0.12	c	0.93	±	0.03	a	1.14	±	0.01	b
C17:0	0.24	±	0.01	0.65	±	0.03	0.25	±	0.03	b	0.22	±	0.01	a	0.21	±	0.01	a	0.20	±	0.01	a
C16:3n-4	0.15	±	0.00				0.11	±	0.09	a.b					0.17	±	0.01	a	0.04	±	0.07	a.b
C18:0	5.10	±	0.11	1.37	±	0.16	9.11	±	0.39	c	10.61	±	0.30	b	7.42	±	0.23	a	7.44	±	0.19	a
C18:1n-9	25.31	±	0.31	1.98	±	0.68	23.93	±	0.21	b	25.15	±	0.17	b	29.17	±	0.19	a	25.29	±	0.54	b
C18:1n-7	1.76	±	0.03	0.56	±	0.05	0.74	±	0.03	a.b	0.77	±	0.08	b	0.66	±	0.06	a	0.66	±	0.06	a
C18:2n-6 (ω-6)	44.49	±	0.37	62.56	±	1.03	35.04	±	1.04	b.c	33.74	±	0.53	a.c	33.59	±	0.52	a	35.34	±	0.55	b
C18:3n-3 (ω −3)	4.09	±	0.15	6.13	±	0.11	1.54	±	0.10	b	1.60	±	0.06	b	1.24	±	0.04	a	1.38	±	0.03	a.b
C20:0	0.33	±	0.01	0.96	±	0.09	0.17	±	0.00	c	0.29	±	0.02	b	0.45	±	0.04	a	0.35	±	0.02	a.b
C20:1n-9	0.48	±	0.01	0.09	±	0.16					0.05	±	0.08		0.10	±	0.08					
C20:1n-7	0.13	±	0.12																			
C20:2n-6 (ω −6)	0.15	±	0.13	0.08	±	0.13																
C20:3n-6 (ω −6)															0.31	±	0.04					
C22:0	0.30	±	0.02	1.72	±	0.05																
C22:1n-9	0.21	±	0.01	0.33	±	0.33																
C24:0	0.22	±	0.01	1.17	±	0.09																
C22:6n-3 (DHA. ω −3)							0.35	±	0.04													
unknown	0.01	±	0.01	2.96	±	0.15	0.67	±	0.19		0.37	±	0.02		0.74	±	0.47		2.23	±	0.24	
SFA	22.56	±	0.34	25.31	±	0.47	36.36	±	1.32	b	36.53	±	0.56	b	32.75	±	0.13	a	33.38	±	0.71	a.b
MUFA	28.55	±	0.31	2.96	±	1.10	25.94	±	0.22	c	27.76	±	0.10	b	31.20	±	0.17	a	27.64	±	0.60	b
PUFA	48.88	±	0.31	68.77	±	1.09	37.03	±	1.19	b	35.34	±	0.57	a	35.32	±	0.50	a	36.75	±	0.48	b
ω −3	4.09	±	0.15	6.13	±	0.11	1.89	±	0.09	c	1.60	±	0.06	b	1.24	±	0.04	a	1.38	±	0.03	a.b
ω −6	44.64	±	0.30	62.63	±	1.02	35.04	±	1.04	a.c	33.74	±	0.53	a	33.91	±	0.49	a	35.34	±	0.55	b.c
ω −6/ ω −3	10.91			10.22			18.54				21.09				27.35				25.61			

[1] ANOVA at a 5% significance level with a post-hoc Tukey's test was used to investigate significant differences among samples. When equality of variances was not met, Games Howell post-hoc test was used.
[2] Different superscripts in a row denote significant differences among cricket species at a 5% significance.

had the same origin as C18:1n-9.

Only house cricket oil contained DHA (C22:6n-3), suggesting elongation and desaturation of C18:3n-3. Blomquist et al. (1991) reported conversion of diet derived C18:3n-3 to 20:3n-3, 20:4n-3 and 20:5n-3. In previous studies, EPA (C20:5n-3), but not DHA, was reported in the house cricket (Kulma et al., 2019; Oonincx et al., 2019; Tzompa-Sosa et al., 2014). Although, no evidence of further elongation to C22 FAs was reported in Australian field cricket, EPA is a precursor of DHA and hence share the same pathway. It is plausible that further elongation from EPA exists in these cricket species but this needs to be further explore. Increased intake of EPA and DHA reduces the risk of coronary diseases, however, this risk reduction can also be achieved by lowering the ω-6/ω-3 FA ratio (≤5) (Simopoulos, 2008). These four cricket species have a far higher ω-6/ ω-3 FA ratio (>18/1) which can lead to adverse health consequences. This high ratio is due to the high content of C18:2n-6 in the feed which is retained in the crickets. It worth noticing that C18:2n-6 is also an essential FA for humans but an excess may adversely affect the brain (Taha, 2020). If these cricket oils are intended for human consumption, it will be pertinent to decrease the ω-6/ ω-3 FA ratio, which could be achieved by including ω-3 PUFAs in cricket diets (Oonincx et al., 2019).

3.2. Phospholipid profile

This high-throughput screening technique allows detection of phospholipid species including its saturation level. In total, 211 phospholipids corresponding to eight phospholipid types, with up to eight unsaturation points, were identified in these four cricket species (Table S1). The identified phospholipids were phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylinositol (PI), sphingomyelin (SM), phosphatidylglycerol (PG), hexosylceramides (HexCer) and lactosylceramide (LacCerd). The most abundant being PC, followed by PS, PE and PI (Fig. 1). These four phospholipids accounted for about 83% of the total relative abundance of phospholipids.

Small differences in relative abundance among cricket species were found when each phospholipid type was individually compared (Fig. 1). No significant differences ($p > 0.05$) were found in SM and HexCer. In the other six phospholipid types significant differences were found. However, the variation in abundance among species was small (<2.2% abundance). Greater differences were observe when the overall phospholipid profile was studied by PCA. This multivariate analysis allowed the recognition of differences among cricket species. The data matrix consisted of 12 samples and 211 variables corresponding to the relative abundance of the identified phospholipids. The first two principal components explained approximately 53.6% of the variance. From the visual inspection of the PCA (Fig. 2), it is observed that Jamaican field crickets and two-spotted crickets, which both belong to the same genus (*Gryllus*), have a similar profile which is increased in PC40:1, PC42:4, PC42:5 and PI38:4. Banded crickets differ from the other species and have increased levels of PC 38 with one, four and six unsaturation levels, PC40:6 and SM24:6. Finally, house crickets differ from the other species with increased levels of PC38 with two and three unsaturation points, PC42 with two, six and eight unsaturation points and SM 20:2. Because the four species were reared under identical conditions, the differences in phospholipid species among crickets could be related to FA preference of the enzymes involved in esterifying FA within the glycerol backbone.

3.3. Identification and quantification of non-polar lipids

In this study identification of all non-polar lipids (DAG, waxes, sterols, TAG) was performed by MALDI-TOF MS. Complimentary quantification of TAG and sterols was performed to gain insight on the complexity of cricket oils. MALDI-TOF MS is a powerful and fast technique for lipid identification and fingerprinting. However, quantification is not possible. TAG and sterols were identified and quantified accurately by combining MALDI-TOF fingerprint with quantification by HPLC-ELSD, GC-FID and GC–MS.

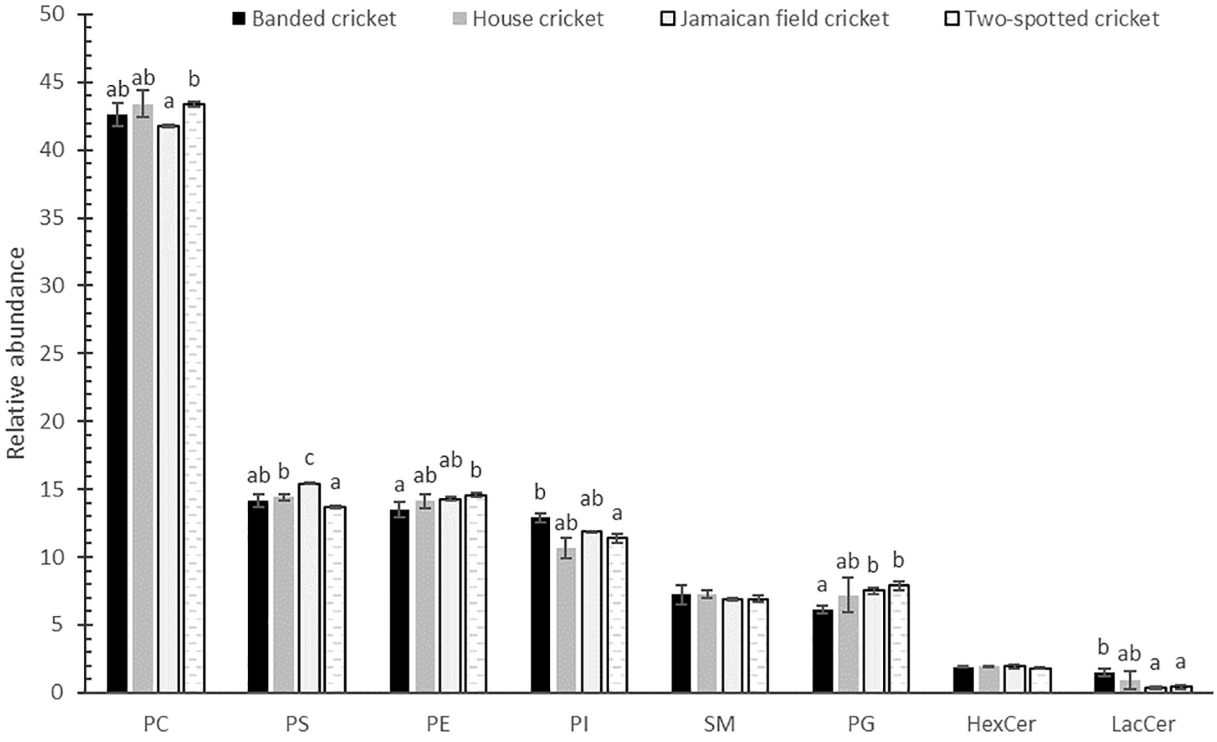

Fig. 1. Phospholipids in cricket lipid extraction as profiles by LC MS. The mean difference is significant at the 0.05 level and are indicated by different letters. Abbreviations: PC- phosphatidylcholine, PS- phosphatidylserine, PE- phosphatidylethanolamine, PI- phosphatidylinositol, SM- sphingomyelin, PG- phosphatidylglycerol, HexCer- Hexosylceramides, LacCer- Lactosylceramide.

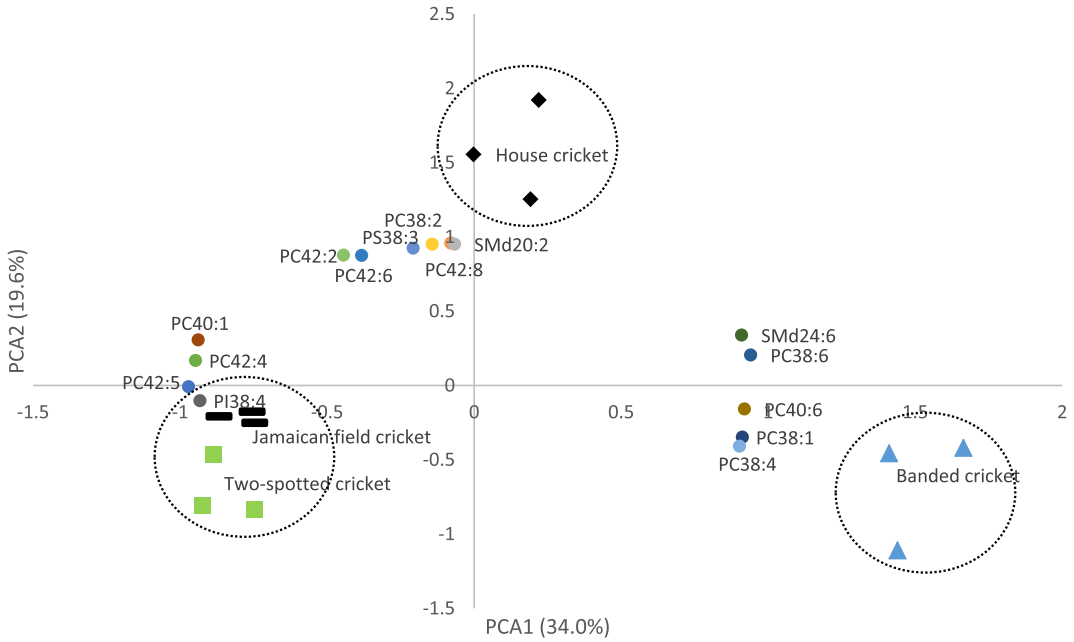

Fig. 2. Principal component analysis (scores and loadings) showing differences in phospholipid species of cricket species measured by liquid chromatography. For the sake of clarity only scores loadings > 0.9 for PCA1 and > 0.85 for PCA 2 are shown.

3.3.1. Non-polar lipid identification by MALDI-TOF MS.

This high-throughput screening technique allows detection of several lipid types, such as waxes, DAG and sterols. We identified 94 masses within the range 403 to 943 *m/z* (Supplement Table S2). An example of the partial positive-ion MALDI-TOF spectrum is shown in Fig. S2 in the supplement section. The low mass range (400–700 *m/z*) corresponded to waxes, DAG and sterols. Some masses corresponded to more than one lipid specie, for this reason some masses have more than one lipid assign. Nonetheless, we uniquely identify five masses corresponding to wax esters, seven masses corresponding to DAG and two to sterols. Moreover, several esterified sterols and waxes were tentatively identified, meaning that also other lipid species have the same mass. In crickets, waxes are structural lipids mainly found in their cuticle (Hendricks & Hadley, 1983). DAG can be intermediaries of TAG synthesis or could be formed from TAGs due to lipase activity during extraction. Sterols, however, are either blood derived or are partial products of 24-dealkylation pathway converting dietary phytosterols to cholesterol (Behmer & David Nes, 2003).

The higher mass range of these cricket lipids (>740 *m/z*) corresponded uniquely to TAG. As expected, this was the most varied non-polar lipid in cricket oil. We identified fifty-one TAGs with carbon numbers (CN; sum of the carbons in the three FA) between 42 and 57 and up to nine unsaturation points. The TAG with the highest relative abundance was CN52:3 (~8.7%), which could correspond to the TAG 16:0–18:2–18:1 (PLO). The second most abundant TAG on a relative basis was CN52:2 (~7.7%), which corresponds to the TAG 16:0–18:1–18:1 (POO) and 16:0–18:2–18:0 (PLS). The third and fourth most abundant TAG on a relative basis were CN50:1 (~5.9%), and CN50:2 (~5.8%), which correspond to the TAG 16:0–16:0–18:1 (PPO) and 16:0–18:2–16:0 (PLP), respectively. The TAG CN54:4 (~4.9%) and CN52:2 (~4.8%), were also abundant in these oils. The corresponding TAG could be 18:2–18:1–18:1/ 18:2–18:2–18:0 (LOO/LLS)) and 16:0–18:2–18:2 (PLL), respectively. This tentative FA identification is plausible because 16:0, C18:0, C18:2, and C18:1 FA account for > 92% of the total FA in this oil. A previous study profiling TAG by GC FID in house cricket also reported CN50-54 as the most abundant TAG (Tzompa-Sosa et al., 2014).

3.3.2. TAG profile by HPLC-ELSD

Using HPLC-ELSD we identified 25 TAG from 42 to 56 equivalent carbon number (ECN = CN-2double bond) (Table 2). The most abundant TAG were PLO, POO, PLP, PPO, PLL, LOO, OLL, PLS, SLO and PLS accounting for > 80% of the total TAG in these oils. These TAGs were also identified as highly abundant by MALDI-TOF MS. The TAG profile of cricket oil is different from other animal and vegetable fats and oils as it has a distinctively high amount of PLO and other polyunsaturated TAG (~82% of total TAG) (Table 3), which is atypical for animal-derived lipids. The relative concentration of POO in the cricket oils in our study (~9.7% of total TAG) is similar to almond oil (~9% of total TAG), peanut oil (10.7–11.8% of total TAG), lard (5.4% of total TAG) and cod liver oil (7% of total TAG) (Indelicato et al., 2017; Rohman et al., 2012). The third most abundant TAG in cricket oils; PLP (8.7% of total TAG), is also present in similar relative concentrations in linseed oil (10.8% of total TAG), lard (5.1% of total TAG) and chicken fat (8.4 %of total TAG). As seen in FA and phospholipid profiling, significant differences were seen among cricket species. However, the differences in concentration are minor.

The FA distribution within the TAG has a unique distribution per species. Organisms closely related are more similar than others far away in the evolutionary tree (Smiddy, Huppertz, & van Ruth, 2012) due to similarities in TAG synthesis pathways and enzyme activity and preference. The similarity between species and the unique distribution of FA within the TAG can be tested by comparing random against experimental TAG concentration. The crickets under study showed a non-random FA distribution indicated by the difference between the random and the experimental TAG concentration (Table 2). For instance, the calculated random concentration of LLL in house cricket was 4.3% however the experimental concentration seen was decreased by almost three folds (1.5%). This deficit of linoleic acid was possibly esterified in PLO where the experimental concentration (22.3%) exceeded in 1.6 folds the calculated random concentration (14.2%). This reveals that the enzymes involved in the TAG synthesis pathway in cricket species have a preference to esterify linoleic acid together with palmitic and oleic acid. Overall, the difference between random and experimental TAG among cricket species varies in the same direction except for LOO, POS, PLS, and SOO (Fig. S3).

The relevance of defining TAG profile of cricket species lies in its

Table 2
Experimental and random triacylglycerol composition in (g/100 g of oil) of insect lipids separated by HPLC-ELSD (N = 3)[1,2].

RT (min)	TAG			House cricket *(Acheta domestica)*				Banded cricket *(Gryllodes sigillatus)*				Two-spotted cricket (*Gryllus bimaculatus*)				Jamaican field cricket (*Gryllus assimilis*)			
	CN[3]	ECN[4]	TAG specie	Random	Experimental			Random	Experimental			Random	Experimental			Random	Experimental		
3.7			u.i.*		0.41	±	0.1		0.44	±	0.1		0.14	±	0.0		0.15	±	0.0
4.6			u.i.*		0.17	±	0.1		0.21	±	0.0		0.05	±	0.0		0.08	±	0.0
4.7			u.i.*		0.56	±	0.1		0.66	±	0.1		0.27	±	0.0		0.24	±	0.0
4.9			u.i.*		0.25	±	0.1		0.27	±	0.0		0.03	±	0.0		0.13	±	0.0
5.1			u.i.*		0.65	±	0.2		0.63	±	0.1		0.19	±	0.0		0.26	±	0.0
5.8			u.i.*		0.13	±	0.0		0.04	±	0.0		0.04	±	0.0		0.05	±	0.0
6.3			u.i.*		0.30	±	0.1		0.31	±	0.1		0.04	±	0.0		0.14	±	0.0
6.5			u.i.*		0.68	±	0.1		0.69	±	0.1		0.11	±	0.1		0.23	±	0.0
7.0			u.i.*		1.01	±	0.1		0.67	±	0.1		0.33	±	0.1		0.60	±	0.0
10.3			u.i.*		0.06	±	0.1		0.14	±	0.0		0.11	±	0.0		0.13	±	0.0
12.5	54:6	42	LLL	4.3	1.48	±	0.4	3.8	1.60	±	0.1	3.8	1.55	±	0.0	4.6	1.62	±	0.1
12.9	54:6	42	OLLn	0.78	0.43^{a}	±	0.1	0.8	0.60^{b}	±	0.0	0.7	0.59^{b}	±	0.0	0.8	0.67^{b}	±	0.0
13.8	52:5	42	LLPl	0.34	0.48^{b}	±	0.1	0.5	$0.49^{b.c}$	±	0.0	0.4	0.35^{a}	±	0.0	0.5	0.59^{c}	±	0.0
14.5	52:5	42	PLLn	0.93		±		0.9		±		0.7	0.11^{a}	±	0.0	0.8	0.69^{b}	±	0.0
15.2	54:5	44	OLL	8.76	5.76^{a}	±	1.0	8.4	$6.25^{a.b}$	±	0.4	9.9	7.52^{b}	±	0.1	9.8	$6.92^{a.b}$	±	0.1
15.7	54:5	44	PlOL/PlLP/OLnP	–	0.36^{b}	±	0.0	–	$0.51^{a.b}$	±	0.2	–	0.64^{a}	±	0.0	–	0.61^{a}	±	0.0
16.2	52:4	44	PLL	10.49	$8.72^{a.b}$	±	0.5	9.0	7.44^{a}	±	0.5	8.9	7.97^{a}	±	0.0	10.4	9.62^{b}	±	0.0
16.7	54:4	46	LLS	3.31	1.29^{a}	±	0.2	3.5	1.54^{a}	±	0.1	2.5	1.39^{a}	±	0.1	2.9	1.84^{b}	±	0.0
17.7		46	u.i.	–	0.44	±	0.1	–	0.41	±	0.0	–	0.33	±	0.0	–	0.52	±	0.4
18.3	54:4	46	LOO	5.94	5.37^{a}	±	0.5	6.2	5.96^{a}	±	0.2	8.5	8.68^{c}	±	0.0	7.0	7.12^{b}	±	0.0
19.3	52:3	46	PLO	14.23	$22.26^{a.b}$	±	0.3	13.3	21.22^{b}	±	0.6	15.4	23.68^{a}	±	0.1	14.8	24.83^{a}	±	0.0
19.8	50:2	46	PlOP	0.37	0.54^{a}	±	0.1	0.6	0.92^{b}	±	0.0	0.5	0.88^{b}	±	0.7	0.5	0.94^{b}	±	0.1
20.5	50:2	46	PLP	8.52	10.09	±	1.4	7.1	7.88	±	0.1	7.0	7.94	±	0.1	7.9	9.05	±	0.4
21.4	54:3	48	OOO	1.34	0.69^{a}	±	0.1	1.5	$0.71^{a.b}$	±	0.0	2.5	1.21^{c}	±	0.3	1.7	0.81^{b}	±	0.0
22.2	54:3	48	SLO	4.49	5.00^{a}	±	0.3	5.2	6.31^{b}	±	0.2	4.3	5.56^{a}	±	0.1	4.1	5.08^{a}	±	0.2
22.6	52:2	48	POO	4.83	8.98^{b}	±	0.2	4.9	8.31^{a}	±	0.2	6.7	12.04^{d}	±	0.1	5.3	9.81^{c}	±	0.0
23.3	52:2	48	PLS	5.38	5.90^{b}	±	1.2	5.6	6.74^{c}	±	0.3	3.9	4.03^{a}	±	0.2	4.3	4.22^{a}	±	0.8
23.8	50:1	48	PPO	5.78	9.78^{b}	±	0.2	5.3	7.55^{a}	±	0.1	6.0	$8.63^{a.b}$	±	0.2	5.6	$8.07^{a.b}$	±	0.0
25.3	54:2	48	SOO	1.52	2.09^{c}	±	0.1	1.9	$1.07^{a.b.c}$	±	0.5	1.9	0.64^{a}	±	0.2	1.4	1.29^{b}	±	0.0
25.7	48:0	48	PPP	2.31				1.9	0.74^{a}	±	0.1	1.8	0.89^{b}	±	0.0	2.0			
26.3	54:2	50	SLS	0.85	0.87^{a}	±	0.2	1.1	1.58^{b}	±	0.2	0.5	0.61^{a}	±	0.0	0.6	0.63^{a}	±	0.0
26.8	52:1	50	POS	3.65	3.41^{b}	±	0.0	4.1	5.46^{c}	±	0.4	3.4	2.60^{a}	±	0.0	3.1	2.22^{a}	±	0.4
27.3		50	u.i.	–	0.34	±	0.0	–				–				–			
28.2	50:0	50	PPS	2.19	0.59^{b}	±	0.0	2.2	0.76^{c}	±	0.1	1.5	0.26^{a}	±	0.0	1.6	0.24^{a}	±	0.1
29.5	54:1	52	SOS	0.58	$0.20^{a.b.c}$	±	0.0	0.8	$0.30^{b.c}$	±	0.0	0.5	$0.19^{a.b.c}$	±	0.0	0.4	$0.15^{a.b}$	±	0.2
30.4	52:0	52	PSS	0.69	0.03^{a}	±	0.1	0.9	0.49^{b}	±	0.1	0.4	0.06^{a}	±	0.0	0.4			
31.2	56:1	56	SOAr	0.02	0.12	±	0.1	0.0	0.15	±	0.0	0.1	0.02	±	0.0	0.0			
32.2			u.i.	–	0.05	±	0.0	–	0.39	±	0.0	–				–	0.04	±	0.0
	Monounsaturated TAG	13.51				13.46				11.44				10.44					
	Polyunstaurated TAG	80.31				79.13				85.28				85.65					
	Saturated TAG	0.62				1.99				1.21				0.24					
Total				91.6	99.5			89.5	99.08			91.7	99.7			90.5	99.6		

* Possibly a diacylglycerol
[1] ANOVA at a 5% significance level with a post-hoc Tukey's test was used to investigate significant differences among samples. When equality of variances was not met, Games Howell post-hoc test was used.
[2] Different superscripts in a row denote significant differences among cricket species at a 5% significance.
[3] CN- carbon number
[4] ECN- equivalent carbon number. ECN = CN- 2 double bond

Table 3
Sterols composition in four cricket species as analyzed by GC-FID (mg/100 g of oil) percentage of total sterols in parenthesis (N = 2).

Organism		Cholesterol	Campesterol	Stigmasterol	Beta-sitosterol	Others	Total phytosterols	Total sterols
Grillidae familiy (crickets)	*House cricket (Acheta domestica)*	1510.81 (82.5)	54.78 (3)	170.94 (9.3)	94.74 (5.2)	–	320.46 (17.5)	1831.27
	Banded cricket *(Gryllodes sigillatus)*	1238.16 (84.2)	63.86 (4.3)	73.2 (5)	94.59 (6.4)	–	231.65 (15.8)	1469.81
	Two-spotted cricket (*Gryllus bimaculatus*)	1025.57 (91.2)	32,053 (2.9)	–	66.18 (5.9)	–	98.71 (8.8)	1,124,028
	Jamaican field cricket (*Gryllus assimilis*)	1358.66 (91.5)	41.2 (2.8)	11.18 (0.8)	73.5 (5)	–	125.88 (8.5)	1484.54
Vegetable oils	Corn[1]	–	200.5	67.7	645.7	10.4	924.3 (100)	924.3
	Palm[1]	–	13.9	9.5	42.6	3.3	69.3 (100)	69.3
	Palm olein[1]	–	19.5	10.5	51	–	81 (100)	81
	Peanut[1]	–	37.8	21.9	169	–	228.7 (100)	228.7
	Sunflower[1]	–	41	33.7	265.3	43.2	383.2 (100)	383.2
Animal fat	Milk fat[2]	222–420	–	–	–	–	–	222–420
	Eggs[3]	400–405	–	–	–	–	–	400–405
	Fish oil	570					–	570
Insects[4]	*Oryctes rhinoceros* larvae	36.6	–	–	–	–	–	36.6
	Imbrasia belina larvae	31.3	–	–	–	–	–	31.3
	Macrotermes bellicosus	41.8	–	–	–	–	–	41.8
	Rhynchophorus phoenicis	34.4	–	–	–	–	–	34.4

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several insect physiological implications and industrial applications. Knowledge on TAG profiles is hence necessary to better understand lipid metabolism (Innis, 2011), interspecies differences (Smiddy et al., 2012) and to understand the effect of animal feeding regimes and genetics on lipid synthesis (Tzompa-Sosa et al., 2018). Moreover, it is useful in the food industry as a quality control parameter to determine adulterations (Indelicato et al., 2017), and to predict physical properties of fats and oils, such as melting and crystallization characteristics (Sato & Ueno, 2014).

3.3.3. Sterols profile by GC-FID and GC–MS

Seven sterols were identified via GC–MS in the cricket oils; namely cholesterol, stigmasterol, β-sitosterol, desmosterol, campesterol, cholestan − 3 – ol and cholest-7-en-3-ol (Supplements Fig. S4). These sterols were identified in all samples except for stigmasterol, which was absent in Two-spotted cricket. Previous studies also identified cholesterol, campesterol and beta-sitosterol in house cricket, (Jing & Behmer, 2020) they did not identified all other sterols present in our study but they do report two other sterols (cholecalciferol and lanosterol). The differences could be related to the extraction method. Contrary to most other animals, insects require a dietary source of sterols (Blomquist et al., 1991). Hence, the sterols identified in the cricket oils are either derived directly from the feed, such as stigmasterol, β-sitosterol and campesterol, or are metabolites of these feed sterols, such as desmosterol, cholesterol, cholestan − 3 – ol and cholest-7-en-3-ol. Cholesterol can be converted to 7- dehydrocholesterol, which is the moulting hormone precursor (Behmer & David Nes, 2003), as well as a vitamin D_3 precursor (Oonincx et al., 2018).

The house cricket is able to convert β-sitosterol and stigmasterol via several intermediates to cholesterol (Behmer & David Nes, 2003). This in turn can be converted to cholestan-3-ol (Behmer & David Nes, 2003; Jing & Behmer, 2020), which is subsequently converted to cholest-7-en-3-ol, also known as lathosterol, and is a component of animal cell membranes (Behmer & David Nes, 2003).

Using GC-FID we quantified cholesterol, campesterol, stigmasterol and β-sitosterol in the lipid extracts (Table 3). Cholesterol was the dominant sterol in these cricket oils, accounting for ~87% of the total sterols. This concurs with Martin and Carls (1968), which reported ~84% of free sterols in house crickets being cholesterol, as is common in most insect species (Jing & Behmer, 2020). House crickets had the highest concentration of cholesterol and phytosterols, whereas these sterols were the lowest in banded crickets. Jamaican field cricket and two-spotted cricket showed a similar concentration of cholesterol and phytosterols. The cholesterol concentration found in these cricket oils is several folds higher than that found in other typical animal fats and in other insects (Table 3). It is recognized that these sterols are present in crickets but, to our knowledge, no sterol quantification has been previously reported. Hence, no quantitative comparison across studies can be made. If these oils are used as food ingredient, it is advisable to partly remove cholesterol to improve its nutritional quality.

High-throughput screening techniques for fingerprinting and well-stablished chromatographic techniques for quantification of phospholipids, TAG and sterols in cricket oils and in general in insect fats and oils is scarce mainly because the edible insect industry is in an early stage of development. Moreover, little attention has been given to the nutritional value and valorization of insect oils and fats. This study offers an overview of the lipidome variation of the most relevant cricket species used as food and feed which is only related to its genetic differences, since the rearing conditions were constant through species.

3.4. Potential applications of cricket oil lipid profile

The FA profiles of insect oils have been described as being in-between vegetable oils and animal fats mainly because of the high concentrations of C16:0, unsaturated C18 FA and the presence of ω-3 FA (Tzompa-Sosa & Fogliano, 2017). The crickets in our study also follow this pattern. These cricket oils are liquid at room temperature despite its > 30% of SFA in the oil. This is explained by the fact that SFA are accommodated within the TAG molecule with UFA forming mixed-acid TAG. The presence of mix-acid TAG causes decrease in its melting point, since this hinders FA chain-chain interaction impairing crystallization (Sato & Ueno, 2014). Regarding food applications, the large amount of UFA (63–66% of FA) will make this oil inappropriate as a frying oil, but it might be suitable as a dressing oil. Further research on cricket oils as food should be undertaken to investigate their sensorial attributes, oxidation stability, and the presence of antioxidant compounds to

determine their shelf life, in this way the full value of these oils as a food ingredient could be assess.

The phospholipid fractions of our cricket oils are rich in PC, PE, and PI, which are the main components of lecithin (Szuhaj, 2003). Lecithin is a surfactant used in food as an antioxidant, flavor protector and emulsifier in a wide range of food products and it is typically extracted from crude soybean oil (Szuhaj, 2003). However, there is a search for alternative sources due to consumer reluctance to soy lecithin because of low traceability, use of GMO soybeans and increased allergic reactions to soybean. In this regard, crickets are a promising alternative source of lecithin. Research on extraction, and functional characterization of cricket lecithin is needed to properly assess its applications in food systems, pharmaceuticals or cosmetics.

Regarding nutritional quality, house crickets were the only species with detectable levels of the long chain ω-3 FA DHA. Although present in small concentrations (0.35% of TFA), this indicates that this species can synthesize *de novo* this FA. The presence of phytosterols in cricket oil could bring health benefits since they are bioactive compounds. Phytosterols are associated with lower levels of LDL cholesterol and reduce intestinal cholesterol absorption (Grille et al., 2010). Moreover, phytosterols are used as food additives (E499) and are used to induce structuring oils into oleogels, which are alternatives to saturated and trans-fatty acids in lipid-based food products (Bin Sintang et al., 2020).

4. Conclusions

The present work gives a detailed profile of the non-polar lipid fraction of four cricket species and highlights the variation between these species. This study reveals the complexity of their lipid profiles, which is typical of animal fats. We hypothesized that lipid metabolism would differ among species of the Gryllidae family, affecting both the lipid content and its profile. Lipid content was indeed greatly affected. However, in general, the profiles of the four cricket species were rather similar. The FA and TAG present in the cricket oil were mainly mono- and polyunsaturated. The high ratio between ω-6/ω-3 FAs is undesirable in edible oils, and could be improved by feeding the crickets more ω-3 FA, or less ω-6 FAs. Both cholesterol and several phytosterols were present in the cricket oils. The phytosterols are desirable because they are bioactive compounds. However, the high cholesterol concentration are a point of concern. The FA and TAG profile makes these cricket oils liquid at room temperature with a possible application as a dressing oil. Further research is necessary to investigate oxidation stability and the presence of antioxidant compounds.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Part of this study was conducted at Food Quality and Design group at Wageningen University, while the corresponding authors was ascribed to this institution. The authors wants to thank for the resources used at that research group.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.129077.

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