

Insect-based products commercialized online: a snapshot of lipid oxidation and mineral content

Stefania Balzan,* Luca Fasolato,* Federico Fontana, Sarah Currò, Enrico Novelli

Department of Comparative Biomedicine and Food Science, University of Padua, Legnaro, Italy

*Equal contribution, co-first authors

Correspondence: Sarah Currò, Department of Comparative Biomedicine and Food Science, University of Padua, Viale dell'Università 16, 35020, Legnaro, Italy.
Tel.: +39049 8272846 - Fax: +390498272973.
E-mail address: sarah.curro@unipd.it

Key words: lipid oxidation, heavy metals, mineral elements, food safety, shelf-stable food.

Contributions: SB, conceptualization; data curation; formal analysis; funding acquisition; investigation; writing - original draft; writing - review & editing; LF, conceptualization; data curation; formal analysis; funding acquisition; investigation; writing - original draft; writing - review & editing; FF, data curation; formal analysis; SC, data curation; formal analysis; investigation; writing - original draft; writing - review & editing. EN, conceptualization; data curation; funding acquisition; investigation; writing - original draft; writing - review & editing. All authors read and agreed to the published version of the manuscript.

Conflict of interest: the authors declare that there is no conflict of interest.

Ethics approval and consent to participate: not applicable.

Funding: this work was supported financially by DOR 2016 - Università degli Studi di Padova.

Availability of data and materials: data and materials are available from the corresponding author upon request.

Acknowledgments: the authors wish to thank Simone Moroso, who worked and participated with great enthusiasm.

Received: 21 July 2023.

Accepted: 23 May 2024.

Early access: 6 August 2024.

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0).

©Copyright: the Author(s), 2024

Licensee PAGEPress, Italy

Italian Journal of Food Safety 2024; 13:11600

doi:10.4081/ijfs.2024.11600

Publisher's note: all claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article or claim that may be made by its manufacturer is not guaranteed or endorsed by the publisher.

Abstract

This research aims to monitor the conservation status of the lipid and mineral contents of four shelf-stable insect-based products (yellow mealworm, house cricket, mole cricket, and silkworm) marketed online. A total of 32 single-species packs were purchased from various online commercial suppliers. Moisture, lipids, fatty acids, titratable acidity, mineral elements, and primary and secondary lipid oxidation products were determined. Statistical multivariate approaches were applied to investigate the contribution of each chemical variable to the characterization of edible insects. Titratable acidity (up to 37.3 g oleic acid per 100 g of crickets), as well as primary and secondary lipid oxidation products, showed great variability within and between species. The study revealed a significant occurrence of rancidity (45.5% of the samples exceeded the peroxide limit of 10 mEqO₂/kg; 100% of the samples exceeded the indication of 1 mg/kg malondialdehyde), whereas the heavy metal content indicated a relatively safe condition, suggesting the absence of potential risks to human health. Both the chemical and the elemental properties could be regarded as potential characteristics suitable for authenticating this food matrix. This study contributes to the description of several chemical features in commercialized processed insect-based products, aiming to highlight possible safety concerns and identify those unfit for human consumption.

Introduction

Edible insects represent an opportunity to enhance global food security (Zielińska *et al.*, 2015). Dehydrated insect-based products, due to their stability at room temperature, are readily available through online commerce in various forms, such as snacks, biscuits, protein bars, or powders (Kolakowski *et al.*, 2021).

Limited data is available on the hazardous chemicals in processed food made from insects, either originating from primary contamination or as a result of processing and preservation. In detail, the presence and amount of heavy metals in insects are affected by the species, growth stage, and concentration in the substrate (EFSA, 2015). However, the manufacturing process can also affect the chemical composition and quality of food made of or containing insects (Fombong *et al.*, 2017).

Lipid oxidation is a significant process that takes place during the production and storage of food, leading to various consequences. Oxidation of lipids leads to a decrease in the nutritional value of foods, changes in the flavor, texture, and appearance of foods, a reduction in their shelf life, and significant economic losses. Lipids in foods are easily oxidized, which results in the ingestion of hydroperoxides, aldehydes, ketones, and epoxides [*e.g.*, acrolein, 4-hydroxy-antimonenol, malondialdehyde (MDA), and

cholesterol oxide] (Wang *et al.*, 2023). Primary and secondary oxidation compounds have the potential to affect human health. 4-hydroxy-nonenal, malonaldehyde, epoxides, and hydrogen peroxide by-products are potential carcinogens; hydroperoxides can damage DNA; free radicals can increase oxidative stress; and carbonyl compounds can affect cellular signal transduction (Huang and Ahn, 2019; Wang *et al.*, 2023). In general, fat rancidity is accelerated by the presence of oxygen, trace transition metals, and exposure to light, heat, and humidity, favoring the production of substances with genotoxic and cytotoxic effects (Reitznerová *et al.*, 2017). Polyunsaturated fatty acids (PUFA), due to the presence of double bonds and their higher reactivity, are more susceptible to oxidation than saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) (Tiencheu *et al.*, 2013). Moreover, a deeper investigation concerning the chemical risks associated in general with insects commercialized as food is also required by those individuals involved in risk assessment because they need as much data as possible.

Mealworms (*T. molitor*), crickets (*Acheta domesticus* and *Gryllus campestris*), mole crickets (*Gryllotalpidae* spp.), and silkworms (*Bombyx mori*) are routinely marketed online as edible insects. Regarding their life cycle, mealworms are generally consumed in the larval stage, crickets and mole crickets in the adult stage, and silkworms as pupa (Van Huis *et al.*, 2013; Koc *et al.*, 2014). The aim of this study was to investigate the conservation status of the lipid and mineral contents of four main species of insect-based products marketed online. Although only some of them are authorized as novel foods according to European legislation, the purpose of the study was to provide information and new data for the scientific community, food business operators, and risk managers to support food safety control.

Materials and Methods

Sample collection

The samples analyzed consisted of edible insects that were available for purchase online, without shipping restrictions, by a general consumer.

A total of 32 shelf-stable insect-based product packs (mealworm, cricket, silkworm, mole cricket) were purchased from 9 online commercial suppliers (Fasolato *et al.*, 2018) (Supplementary Table 1). The samples collected covered the widest variability on the market of edible insects (cooked-dehydrated, freeze-dried whole insects, and powder), natural or flavored, at different stages of life and reared on different substrates. Every sample was analyzed before the date of minimum durability (best before). Information about the supply (from catch or rearing) and country of origin was not always provided.

Analytical preparation

The moisture content was determined using a gravimetric method (Fombong *et al.*, 2017). Briefly, samples were crushed with a mortar and incubated at 103°C using a natural air convection oven (MPM Instrument s.r.l. M120 VF, Bernareggio, MB, Italy) until they reached a constant weight. The determination was conducted in duplicate. The lipid extraction procedure was performed using the method proposed by Barcarolo *et al.* (2007), using a solution of dichloromethane/hexane/isopropanol (1:1:1 v:v:v) at a sample:solution ratio of 1:20 (w:v). Fatty acid methyl esters were prepared through acid derivatization using a 3 N methanolic HCl solution, following the protocol outlined by

Fasolato *et al.* (2010), and were subsequently analyzed using gas chromatography (Shimadzu Italia Srl, model G17A, Milano, Italy) with flame ionization detection.

Lipid status

The lipid extract was analyzed to define titratable acidity (TA) and primary [peroxide value (POV)] and secondary lipid oxidation [thiobarbituric acid reactive substances test (TBARS)].

The TA was determined using a lipid aliquot solubilized with a diethyl ether/ethanol mixture (2:1 v:v) and subsequently titrated with 0.01 M sodium hydroxide and expressed as a percentage of oleic acid (Jeon *et al.*, 2016). The POV was determined following the procedure proposed by Bonoli *et al.* (2007) and expressed as mEq O₂ kg⁻¹ fat using a calibration curve made with iron (III) chloride. The TBARS were analyzed as proposed by Botsoglou *et al.* (1994) and expressed as mg of MDA per kg of sample. All measurements were conducted in duplicate, except for the peroxide analysis, which was performed in triplicate.

Mineral composition

The presence of macro (Ca, K, Mg, Na, P, S), micro (Zn, Co, Cr, Cu, Fe, Mn, Mo, Ni), and trace (Cd, Hg, Pb, As) minerals was assessed using inductively coupled plasma optical emission spectrometry (Ciros Vision EOP SPECTRO Analytical Instruments GmbH, Kleve, Germany). In detail, the mineral content was determined after wet digestion of the samples with a mixture of nitric acid and hydrogen peroxide in a microwave oven, with the temperature gradually increasing to 200°C. The concentration range of the calibration solutions was between 0 and 25 mg/L for all the elements.

Data analysis

Due to the small number of samples, the collection methods, and the absence of certain information for some lots (method of production, origin, *etc.*), only non-parametric inferential tests and exploratory distance-based statistics were applied.

With an explorative aim, different multivariate approaches were applied to investigate the contribution of chemical variables to the characterization of edible insects by using different tools of the PRIMER-e platform (<https://www.primer-e.com/>) (Fasolato *et al.*, 2016; McLeod *et al.*, 2016). The insect species was the main factor considered; the chemical data were normalized, and the Gower measure was applied to build the resemblance matrixes. Two different matrixes were prepared; the elemental profiles and chemical data were used as bases for the constrained ordination technique and clustering method, respectively. Hierarchical clustering was applied as an agglomerative approach based on the full linkage method. Non-metric multidimensional scaling plots were used to visualize sample variability, *i.e.*, the dissimilarity between pairs of objects in a two- or three-dimensional space. Canonical analysis of principal coordinates (CAP) was performed primarily to test the influence of the studied factors (species and declared processing method) on sample segregation (McLeod *et al.*, 2016). A leave-one-out cross-validation approach was adopted to evaluate the CAP classification performance. The non-parametric combination (NPC) test was conducted with the free software NPC Test R10 (<http://www.wiley.com/legacy/wileychi/pesarin/material.html>). The partial and global p values were determined for all the investigated features only according to the factor 'insect species'.

Results and Discussion

The results reported in this study provide a cross-section of marketed processed foods containing insects, similar to the study of Kolakowski *et al.* (2021). As reported in *Supplementary Table 2*, no information is available on the country of origin of the insects or the production method (farmed or wild); the results will be analyzed from a consumer perspective, providing only qualitative, descriptive information.

Composition and fat oxidation

The moisture and lipid contents, individual SFA (C4:0-C24:0), MUFA (C14:1-C24:1), and PUFA (C18:2-C22:2) and the FA sum group of the edible insect samples are reported in Table 1. The moisture content was relatively consistent across species, likely because of the particular time-temperature conditions and treatment methods used during insect processing for food production (Fombong *et al.*, 2017). The lipid content and fatty acid profiles of insects can vary significantly based on several factors, *i.e.*, the specific species, life stages, environmental conditions, feeding habits, and methods of lipid extraction (Paul *et al.*, 2017). The lipid composition differed among species (NPC test *p*-value 0.0001); in detail, the silkworm and the mealworm showed the highest average lipid content. Similar results were reported by Zielińska *et al.* (2015) for *Tenebrio molitor*, by Tomotake *et al.* (2010) for *Bombyx mori*, and by Paul *et al.* (2017) for *Acheta domestica*, whereas Yang *et al.* (2006) reported a lower fat content for *Gryllotalpa* spp.

The contents of SFA, MUFA, and PUFA display substantial heterogeneity, both between and within species. Regrettably, the dearth of information on the sourcing of raw materials impedes our comprehension of the underlying factors contributing to these variations. Similar results were observed for *T. molitor* and spent silkworm pupae in a study by Zielińska *et al.* (2015). Nonetheless, significant disparities are noted, specifically for *A. domestica*, compared to the study of Kamau *et al.* (2018), which reported a lower proportion of SFA (27.1%) compared to the findings of the current study. A comprehensive overview of the fatty acids identified in each sample is provided in *Supplementary Tables 2 and 3*.

To monitor the oxidative stability of lipids in the edible insect packs considered in this study, the TA (oleic acid g/100 g), POV (mEq O₂/kg), and MDA (mg/kg) levels were evaluated (Figure 1 A-C, respectively). Similar to other food-producing animals, insects possess lipolytic enzymes that facilitate the hydrolysis of glycerol esters into mono- and di-glycerols, as well as free fatty acids. Furthermore, cold temperatures induce the activation of certain lipases, enhancing glycerol availability (Ma *et al.*, 2020). Consequently, the use of freezing for stunning and euthanasia may also contribute to elevated TA levels. Lipid oxidation is of particu-

lar concern due to its potential to generate detrimental compounds, including free radicals and lipid degradation products, which affect food features (*i.e.*, off-flavors and off-odors, liposoluble vitamin loss, and protein oxidation) and consumer health (development of cardiovascular diseases, chronic inflammation, and other health-related disorders) (Wang *et al.*, 2023; Huang and Ahn, 2019).

In particular, the analysis of TA allowed for the evaluation of the content of free fatty acids, which exhibit greater susceptibility to oxidation compared to their corresponding alkyl esters. The variability of TA according to species is depicted in Figure 1A. In detail, crickets and mealworms exhibited the highest (37.3 and 32.6 oleic acid g/100 g, respectively) recorded TA, approaching that of mole crickets (16.0 oleic acid g/100 g); an increment of TA in oil extracted from mealworms during storage was observed in a study by Jeon *et al.* (2016), whereas silkworms displayed a lower TA average (4.69 oleic acid g/100 g) value, similar to those observed in a study by Escamilla-Rosales *et al.* (2019) regarding dehydrated grasshoppers.

Analysis of POV enabled the measurement of early lipid oxidation products for the species considered in the study (Figure 1B). These products serve as precursors of low molecular weight that contribute to the development of off-flavors or can react with other components present in the food. When we compared our findings to the peroxide limits (20 mEq O₂/kg) of extra virgin and virgin olive oil as specified by European Commission (2011), it was found that 31% of the samples analyzed in this study exceeded the threshold, indicating oxidation. However, considering the stricter limit of 10 mEq O₂/kg suggested by Codex Alimentarius (1997), the percentage of non-compliant samples increased to 45.5%. Tiencheu *et al.* (2013) observed a rise in primary oxidation products (POV) under various conditions, including prolonged refrigeration, freezing at -18°C, dehydration, and specific culinary treatments such as boiling and roasting. Tiencheu *et al.* (2013) attribute this increase to the pro-oxidant action of metals released through protein denaturation and photo-oxidation due to sunlight exposure. Additionally, oxygen is recognized as a crucial factor in lipid oxidation. It is worth noting that an oxygen absorbent was present only in the case of one item in the present study.

The assessment of aldehydes provided valuable insights into the secondary products arising from lipid peroxidation, which are known to contribute to off-flavors, notably the characteristic rancid odor, and play a pivotal role in the biological consequences of lipid oxidation (Tiencheu *et al.*, 2013; Reitznerová *et al.*, 2017; Kamau *et al.*, 2018). Our results revealed higher average levels of MDA in crickets and mealworms, indicating an elevated extent of lipid peroxidation in these specimens. By contrast, silkworms and mole crickets exhibited comparable MDA values (Figure 1C). These findings suggest variations in the degree of lipid oxidation among the different insect species studied and between lots, emphasizing

Table 1. Moisture (g/100 g), fat (g/100 g) and lipid composition (percentage of class of fatty acid over the total identified fatty acids) of processed edible insects.

	Cricket (n=12)			Mealworm (n=10)			Mole cricket (n=3)			Silkworm (n=7)		
	mean±sd	min	max	mean±SD	min	max	mean±SD	min	max	mean±SD	min	max
Moisture	3.5±1.6	1.4	6.0	4.1±2.0	1.6	6.6	3.6±0.9	0,5	5.6	2.1±1.7	2.5	4.2
Fat	18.5±0.7	13.9	23,4	24.9±1.3	19.3	31.3	20.4±1.7	17.8	23.6	31.2±2.2	25.7	37.5
SFA	43.3±4.9	38.5	50.7	29.0±7.7	23.3	45.1	41.4±4.0	38.5	45.9	29.6±2.0	26.4	31.5
MFA	29.0±3.4	24.6	34.0	41.3±7.3	24.0	50.7	42.7±1.6	41.2	44.4	32.3±2.0	24.0	50.7
PUFA	27.1±7.9	17.8	36.8	29.4±5.1	19.6	37.7	15.3±5.2	9.3	18.4	38.0±3.9	34.4	44.1

SD, standard deviation; min, minimum value; max, maximum value; SFA, saturated fatty acids; MFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

the potential impact of species-specific factors on the lipid peroxidation process. Indeed, several studies collectively demonstrated the susceptibility of insects to lipid oxidation and highlighted the potential influence of thermal stress and post-harvest conditions on the oxidative stability of insect-derived products (Jia *et al.*, 2011; Ju *et al.*, 2014; Kamau *et al.*, 2018). Furthermore, the lipid oxidation process is independent of the killing method (Singh *et al.*, 2020) and the effects of storage at ambient temperature on packaged dried crickets (Kamau *et al.*, 2018).

Although a specific limit for secondary oxidation products has not yet been established in Europe, the preservation of fats and liposoluble components remains crucial. Kim *et al.* (2000) and Reitznerová *et al.* (2017) identified threshold limits of MDA for processed catfish, sardines, and meat. In detail, a concentration exceeding 0.5 mg/kg indicated the presence of oxidation; meanwhile, levels exceeding 1-1.1 mg/kg were considered possibly unacceptable for consumption. Furthermore, it is noteworthy that Singh *et al.* (2020) identified *Acheta domesticus* as rancid, with MDA values exceeding 1.6 mg/kg. The value of MDA (crickets:

1.00-8.29 mg/kg; mole crickets: 2.42-3.53 mg/kg; mealworms: 1.70-14.35 mg/kg; silkworms: 1.96-4.40 mg/kg) observed in the present study showed that 100% of the samples were rancid, and the causes could be synergistic and difficult to assign. Nevertheless, it is crucial to acknowledge that oxidation products pose health risks, and it is essential to minimize consumer exposure even before the stage of complete rancidity is reached.

The chemical characteristics enabled the distinct grouping of insect species, as evidenced by the hierarchical clustering and MDS plots. However, the CAP (Figure 2) showed the feasibility of species classification according to the studied variables, with a high classification accuracy (96.5%). The insect species are clearly different with respect to their chemical data profiles and are clearly defined by the CAP analysis. The results highlight the high fat and PUFA concentrations of silkworms, whereas mealworms exhibit elevated MDA and SFA levels. Mole crickets show the highest TA values, whereas other crickets correlate mainly with SFA, such as C18:0 and C22:0. Caution is exercised in the interpretation of these results due to incomplete label data, and cooked/dehydrated sam-

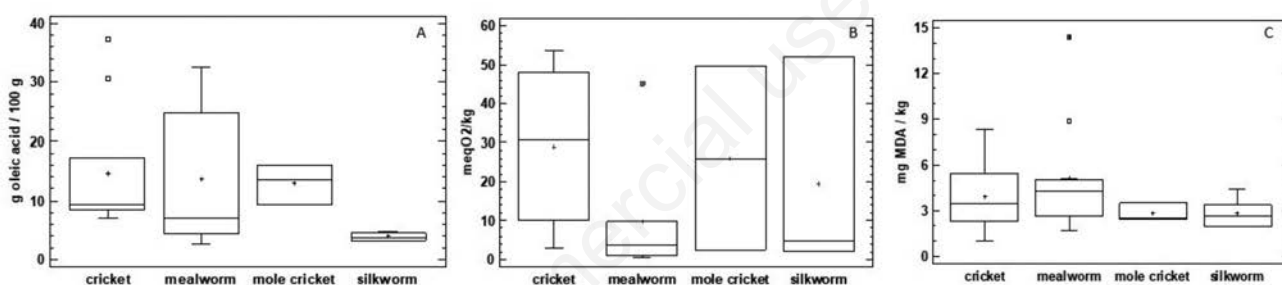


Figure 1. A) Titratable acidity, B) peroxide value; C) malonaldehyde content of processed edible insects. Mealworms (*T. molitor*; n=10), silkworms (*B. mori* n=7), mole crickets (*Gryllotalpidae* spp., n=3) and crickets (*A. domesticus*, n=11; *G. campestris*, n=1). MDA, malonaldehyde.

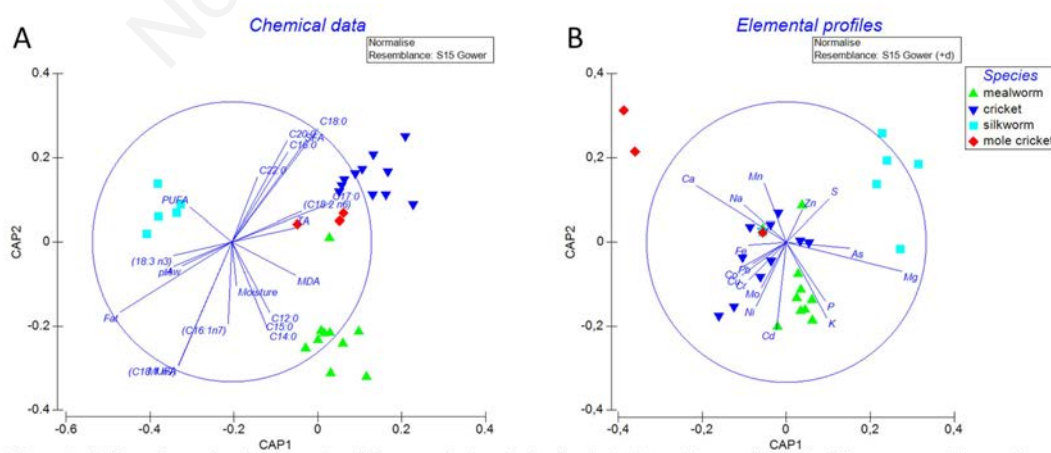


Figure 2. A) Two-dimensional scaling plot of the canonical analysis of principal coordinates of chemical features according to the insect species; B) two-dimensional scaling plot of the canonical analysis of principal coordinates of elemental profiles according to the insect species. Vectors showing the direction of each feature according to Spearman correlation.

ples showed the highest MDA and TA levels, followed by freeze-dried and boiled/dried insects.

Mineral content of insects

The concentrations of heavy metals (Table 2) and minerals (Table 3) exhibited great variability among and between species. Chen *et al.* (2009) reported that edible insects are rich in potassium, sodium, calcium, copper, iron, zinc, manganese, and phosphorus. In the present study, even though they were underrepresented, mole crickets were characterized by a low level of K, Mg, P, and As but were high in Fe, and silkworms showed a low level of Cu and Ni; meanwhile, the maximum level of Na was detected in silkworms and mealworms. This last finding could be ascribed to the use of salt as a flavoring. Crickets were rather similar in mineral composition to mealworms but were characterized by the highest value of Cu. These findings agree with Kolakowski *et al.* (2021), who described this variability as a consequence of species-specific metabolism, the maturity stage, sampling and analytical techniques, environmental contamination, the feeding diet, and, in our case, the small sample number.

Concerning heavy metals, there currently are no specific legal limits for edible insects; therefore, the following considerations

have been made based on the most relevant food categories.

The total Hg content was consistently below the detection limit, compliant with the limit of 0.5 mg/kg for crustaceans, mollusks, and muscle meat of fish indicated by the European Commission (2023). Kolakowski *et al.* (2021) reported very low Hg concentrations (0.0011-0.016 mg/kg), while Kim *et al.* (2015) detected approximately 1.0 ppb in *Gryllus bimaculatus* samples. Factors such as feeding habits, seasons, and the environment affect insect Hg concentrations (Mason *et al.*, 2000). The content of Cd in the present study was below the level considered hazardous for crustaceans (0.50 mg/kg) established by the European Commission (2023); nevertheless, this value was within legal limits. In detail, the maximum level of Cd was found in mealworms (0.22 mg/kg). Alternatively, according to the European Commission (2023), the maximum allowable concentration of Pb is set at 0.50 mg/kg for crustaceans and 1.50 mg/kg for bivalve mollusks. In the current study, when compared to the stricter limit of 0.50 mg/kg, 12.5% of the samples were non-compliant, of which the mole cricket samples exhibited a higher Pb concentration (1.22 mg/kg). However, it is noteworthy that 40.6% of the samples were below the limit of detection. Cd and Pb are the most recurrent metals in the natural environment, arising from natural

Table 2. Heavy metal content of processed edible insects (mg/kg w/w). Results are indicated as mean, minimum, maximum and the number of samples within each species whose values are lower than limit of detection.

	Cricket (n=12)				Mealworm (n=10)				Mole cricket (n=3)				Silkworm (n=7)			
	mean±SD	min	max	n. <LOD	mean±SD	min	max	n. <LOD	mean±SD	min	max	n. <LOD	mean±SD	min	max	n. <LOD
Cd	0.08±0.04	0.01	0.14	5	0.10±0.07	0.01	0.22	2	0.09±0.00	0.09	0.09	2	0.06±0.06	0.02	0.10	4
Hg	<LOD*	-	-	12	<LOD*	-	-	10	<LOD*	-	-	3	<LOD*	-	-	7
Pb	0.41±0.13	0.16	0.53	6	0.29±0.17	0.01	0.48	3	0.94±0.40	0.01	1.22	1	0.32±0.25	0.13	0.60	3
As	0.72±0.61	0.29	1.15	10	1.10±0.74	0.28	1.71	7	<LOD*	-	-	7	1.02±0.50	0.52	1.98	2

LOD, limit of detection; SD, standard deviation; <LOD: n. <LOD, number of sample lower than LOD; min, minimum concentration; max, maximum concentration; <LOD, all samples were lower than LOD; *LOD for As, Pb: 0.01 mg/kg; LOD for Hg, Cd: 0.002 mg/kg.

Table 3. Mineral composition of processed edible insects (mg/kg w/w). Results are indicated as mean, minimum, maximum and the number of samples within each species whose values are lower than limit of detection.

	Cricket (n=12)				Mealworm (n=10)				Mole cricket (n=3)				Silkworm (n=7)			
	mean±SD	min	max	n. <LOD	mean±SD	min	max	n. <LOD	mean±SD	min	max	n. <LOD	mean±SD	min	max	n. <LOD
Ca	1728±1101	389	4005	-	975±1190	281	4220	-	2271±1271	1458	3736	-	1420±1110	829	3681	-
K	6106±3051	1020	9622	-	6764±2138	1481	9197	-	1578±867	584	2184	-	5880±3311	688	8999	-
Mg	766±377	147	1580	-	1589±690	156	2369	-	359±195	146	530	-	2074±986	141	2905	-
Na	2461±796	976	3567	-	3083±2820	853	10114	-	5058±3057	2191	8276	-	6547±9574	187	24720	-
P	6069±1555	3309	7985	-	6235±1551	3491	8143	-	3595±377	3160	3816	-	5541±1344	3115	7015	-
S	3135±944	1412	4033	-	2494±692	1505	4050	-	2690±1163	1374	3581	-	3779±1188	1357	4389	-
Zn	134±61	28.5	208	-	103±45.9	30.5	196	-	90.2±57.8	27.8	142	-	123±56.8	27.5	187	-
Co	0.09±0.03	0.05	0.12	8	0.09±0.03	0.07	0.13	5	0.72±0.15	0.61	0.83	1	<LOD	-	-	4
Cr	0.73±0.75	0.20	2.24	2	0.51±0.55	0.08	1.82	1	0.92±0.20	0.78	1.06	1	0.21±0.09	0.11	0.31	1
Cu	26.5±16.9	0.34	59.9	-	16.9±9.39	0.35	30.3	-	22.8±24.3	0.32	48.5	-	8.38±4.21	0.33	12.5	-
Fe	58.4±36.1	1.20	110	-	47.8±20.8	1.32	76.7	-	264±228	1.09	411	-	34.1±27.9	1.16	85.9	-
Mn	25.6±18.3	0.14	60.4	-	10.9±11.4	0.15	42.3	-	96.2±8.30	0.13	196	-	16.8±9.80	0.14	28.1	-
Mo	0.67±0.42	0.10	1.24	2	0.87±0.37	0.25	1.50	1	1.44±0.06	1.05	1.83	1	0.60±0.30	0.38	0.81	3
Ni	1.03±1.83	0.09	6.14	2	0.88±0.83	0.23	2.80	1	1.16±0.28	0.96	1.36	1	0.17±0.08	0.09	0.24	2

LOD, limit of detection; SD, standard deviation; <LOD: n. <LOD, number of sample lower than LOD; min, minimum concentration; max, maximum concentration; <LOD, all sample.

sources and human activities (industry and agriculture; Kolakowski *et al.*, 2021). Although Zhuang *et al.* (2009) demonstrated decreases in heavy metal accumulation along the soil-plant-insect-chicken food chain, the same authors demonstrated the passage of Pb and Cd from soil to insects (*Spodoptera litura*) and found that subsequent fecal elimination limited bioconcentration.

European Regulation (2023) fixed the maximum levels of inorganic As in foodstuffs. Unfortunately, As was determined as total, it was not possible to differentiate between inorganic and organic arsenic species. In addition, no limits have currently been set for products of animal origin. The results showed that 90.6% of the samples had values lower than the limit of detection, ranging between 0.28 and 1.98 mg/kg. Conversely, the heavy metal levels found disagree with those reported by Poma *et al.* (2017), who analyzed edible insects of European origin. Also, Kolakowski *et al.* (2021) have shown great variability in the concentration of metals; however, they did not highlight the risks to consumer health. Elemental profiles can be applied as fingerprints to identify the origin of food and resolve other authentication issues by the use of CAP (Figure 2B) (McLeod *et al.*, 2016). Elemental profiles were considered a trace marker for species identification; however, leave-one-out cross-validation analysis showed an incomplete class attribution for mealworms, mole crickets, and silkworms (from 66.7% to 83.3% were correctly classified). By contrast, crickets were clearly discriminated against, with 91.7% correct sample attribution. A larger data set in terms of samples and related information would probably improve the classification capabilities.

Conclusions

This study, despite being conducted on a limited number of samples and lacking information about the source soils and substrates of the edible insects, had the advantage of absolute fortuity in the sample collection and allowed us to reach some important conclusions. The study highlights the high susceptibility of some edible insects to lipid oxidation. Rancidity is responsible for alterations in organoleptic traits, nutritional loss, and the accumulation of compounds harmful to human health. Although there are no specific legal limits, it is important to control the degradation processes that affect fats and oils. The farming or harvesting environment represents one of the main sources of hazards. In this regard, the elemental profile showed a fairly reassuring situation that must be kept under control with recurring monitoring. Both chemical and elemental features could be considered putative characteristics suitable for the authentication of this food matrix.

References

- Barcarolo R, Novelli E, Balzan S, Granata A, Antonetti P, 2007. PCB's and hexachlorobenzene determination in fish by isotope dilution and GC-MS negative chemical ionisation mode. Conference presentation at the 25th Informal meeting on mass spectrometry, 6th–10th May, 2007, Nyíregyháza-Sóstó, Hungary, Comm.MoPo30.
- Bonoli M, Caboni MF, Rodriguez-Estrada MT, Lercker G, 2007. Effect of feeding fat sources on the quality and composition of lipids of precooked ready-to-eat fried chicken patties, *Food Chem* 101:1327-37.
- Botsoglou NA, Fletouris DJ, Papageorgiou GE, Vassilopoulos VN, Mantis AJ, Trakatellis AG, 1994. Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid-peroxidation in animal tissue, food, and feedstuff samples. *J Agric Food Chem* 42:1931-7.
- Chen X, Feng Y, Chen Z, 2009. Common edible insects and their utilization in China. *Entomol Res* 39:299-303.
- Codex Alimentarius, 1997. Report of the fifteenth session of the codex committee on fats and oils London, United Kingdom, 4-8 November 1996; Joint Fao/Who Food Standards Programme Codex Alimentarius Commission Twenty-second Session Geneva, 23-28 June 1997, CL 1997/2-FO January 1997. Available from: https://www.fao.org/input/download/report/313/a197_17e.pdf.
- EFSA, 2015. Risk profile related to production and consumption of insects as food and feed. *EFSA J* 10:26-9.
- Escamilla-Rosales MF, Ariza-Ortega JA, Ramos-Cassellis ME *et al.* 2019. Comparison of the proximal chemical and fatty acid composition of the fried grasshopper's (Orthoptera) dish. *Eur Food Res Technol* 245:1629-40.
- European Commission, 2011. Commission Regulation (EU) No 61/2011 of 24 January 2011 amending Regulation (EEC) No 2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. In: *Official Journal, L* 23, 27/01/2011.
- European Commission, 2023. Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006. In: *Official Journal, L* 119/103, 05/05/2023.
- Fasolato L, Cardazzo B, Carraro L, Fontana F, Novelli E, & Balzan S, 2018. Edible processed insects from e-commerce: food safety with a focus on the *Bacillus cereus* group. *Food Microbiol* 76:296-303.
- Fasolato L, Novelli E, Salmaso L, Corain L, Camin F, Perini M, Antonetti P, Balzan S, 2010. Application of nonparametric multivariate analyses to the authentication of wild and farmed European sea bass (*Dicentrarchus labrax*). Results of a survey on fish sampled in the retail trade. *J Agric Food Chem* 58:10979-88.
- Fombong FT, Van Der Borgh M, Vanden Broeck J, 2017. Influence of freeze-drying and oven-drying post blanching on the nutrient composition of the edible insect *Ruspolia differens*. *Insects* 8:102.
- Huang X, Ahn DU, 2019. Lipid oxidation and its implications to meat quality and human health. *Food Sci Biotechnol* 28:1275-85.
- Jeon YH, Son YJ, Kim SH, Yun EY, Kang HJ, Hwang IK, 2016. Physicochemical properties and oxidative stabilities of mealworm (*Tenebrio molitor*) oils under different roasting conditions, *Food Sci Biotechnol* 25:105-10.
- Jia F, Hu F, Wang J, 2011. Effects of thermal stress on lipid peroxidation and antioxidant enzyme activities of oriental fruitfly, *Bactrocera dorsalis* (Diptera: Tephritidae). *Fla Entomol* 94:956-63.
- Ju R, Wei H, Wang F, ZHOU XH, LI B, 2014. Anaerobic respiration and antioxidant responses of *Corythucha ciliata* (Say) adults to heat-induced oxidative stress under laboratory and field conditions. *Cell Stress and Chaperones* 19:255-62.
- Kamau E, Mutungi C, Kinyuru J, Imathiu S, Tanga C, Affognon H, Ekesi S, Nakimbugwe D, Fiaboe, KKM, 2018. Effect of packaging material, storage temperature and duration on the quality of semi-processed adult house cricket meal. *J Food Res* 7:21-31.
- Kim E, Lim J, Chang Y, An S, Ahn MY, 2015. Changes in the quality characteristics of cricket (*Gryllus bimaculatus*) under vari-

- ous processing conditions. *Korean J Food Preserv* 22:218-24.
- Kim TJ, Silva JL, Chamul RS, Chen TC, 2000. Influence of ozone, hydrogen peroxide, or salt on microbial profile, TBARS and color of channel catfish fillets. *J Food Sci* 65:1210-3.
- Koc K, Incekara U, Turkez H, 2014. Biomonitoring of the genotoxic effects and oxidative potentials of commercial edible dung beetles (*Onitis* sp.), grasshopper (*Caelifera* sp.) and mole crickets (*Gryllotalpa* sp.) in vitro. *Toxicol Ind Health* 30:683-9.
- Kolakowski BM, Johaniuk K, Zhang H, Yamamoto E, 2021. Analysis of microbiological and chemical hazards in edible insects available to canadian consumers. *J Food Prot* 84:1575-81.
- Ma W, Zhao X, Yin C, Jiang F, Du X, Chen T, Zhang Q, Qiu L, Xu H, Hull JJ, Li G, Sung WK, Li F, Lin Y, 2020. A chromosome-level genome assembly reveals the genetic basis of cold tolerance in a notorious rice insect pest, *Chilo suppressalis*. *Mol Ecol Resour* 20:268-82.
- Mason RP, Laporte JM, Andres S, 2000. Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. *Arch Environ Contam Toxicol* 38:283-97.
- McLeod RJ, Prosser CG, Wakefield JW, 2016. Identification of goat milk powder by manufacturer using multiple chemical parameters. *J Dairy Sci* 99:982-93.
- Paul A, Frederich M, Caparros Megido R, Alabi T, Malik P, Uyttenbroeck R, Francis F, Blecker C, Haubruge E, Lognay G, Danthine S, 2017. Insect fatty acids: a comparison of lipids from three Orthopterans and *Tenebrio molitor* L. larvae. *J Asia-Pac Entomol* 20:337-40.
- Poma G, Cuyx M, Amato E, Calaprice C, Focant JF, Covaci A, 2017. Evaluation of hazardous chemicals in edible insects and insect-based food intended for human consumption. *Food Chem Toxicol* 100:70-9.
- Reitznerová A, Šuleková M, Nagy J, Marcinčák S, Semjon B, Čertík M, Klemková T, 2017. Lipid peroxidation process in meat and meat products: a comparison study of malondialdehyde determination between modified 2-thiobarbituric acid spectrophotometric method and reverse-phase high-performance liquid chromatography. *Molecules* 16:1988.
- Singh Y, Cullere M, Kovitvadhi A, Chundang P, Dalle Zotte A, 2020. Effect of different killing methods on physicochemical traits, nutritional characteristics, in vitro human digestibility and oxidative stability during storage of the house cricket (*Acheta domesticus* L.). *IFSET* 65:102444.
- Tiencheu B, Macaire Womeni H, Linder M, Tchouanguép Mbiapo F, Villeneuve P, Fanni, J, Parmentier, M, 2013. Changes of lipids in insect (*Rhynchophorus phoenicis*) during cooking and storage. *Eur J Lipid Sci Tech* 115:186-95.
- Tomotake H, Katagiri M, Yamato M, 2010. Silkworm pupae (*Bombyx mori*) are new sources of high quality protein and lipid. *J Nutr Sci Vitaminol* 56:446-8.
- Van Huis A, Van Itterbeeck J, Klunder H, Mertens E, Halloran A, Muir G, Vantomme P, 2013. Edible insects: future prospects for food and feed security. Available from: <https://www.fao.org/4/i3253e/i3253e.pdf>.
- Wang D, Xiao H, Lyu X, Chen H, Wei F, 2023. Lipid oxidation in food science and nutritional health: A comprehensive review. *Oil Crop Sci* 8:35-44.
- Yang LF, Siriamornpun S, Li D, 2006. Polyunsaturated fatty acid content of edible insects in Thailand. *J Food Lipids* 13:277-85.
- Zhuang P, Zou H, Shu W, 2009. Biotransfer of heavy metals along a soil-plant-insect-chicken food chain: field study. *J Environ Sci* 21:849-53.
- Zielińska E, Baraniak B, Karaś M, Rybczyńska K, Jakubczyk A, 2015. Selected species of edible insects as a source of nutrient composition. *Food Res Int* 77:460-6.

Online supplementary material:

Supplementary Table 1. Description of insect-based food acquired from commercial online platforms.

Supplementary Table 2. Unsaturated fatty acid (percentage of single fatty acid over the total identified fatty acids).

Supplementary Table 3. Saturated fatty acid (percentage of single fatty acid over the total identified fatty acids).