



Nutritional value, protein and peptide composition of edible cricket powders

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ABSTRACT

There are various indicators, including FAO and EU sources, that edible insects could become one of the solutions to the problem of global food supply. This report was aimed at improving the knowledge on powdered crickets (*Acheta domesticus*). The analyses of the basic nutritional composition revealed that cricket powders were rich in protein (42.0–45.8% of dry matter) and fat (23.6–29.1% of dry matter). In terms of mineral content, CPs were rich in Ca, Mg and Fe. Most of all, the levels of Cu, Mn and Zn were especially high (2.33–4.51, 4.1–12.5, 12.8–21.8 mg/100 g of dry matter, respectively). Furthermore, the analyses into the proteins indicated that the cricket powders were treated with high temperatures and allowed the determination of four cricket-specific peptides that showed sufficient thermostability to serve as markers for authentication.

1. Introduction

It is estimated that by 2050 the world's population will be up to 9 billion. Therefore, one of the global problems may be to feed the population with appropriate amounts of food and in particular with adequate protein supply (FAO, 2012). Global environmental changes generate the need to search for new, unconventional source of protein for human nutrition. For this reason, the Food and Agriculture Organization of the United Nations (FAO) recommends the consumption of edible insects due to their high nutritional value and environmentally friendly breeding conditions (FAO, 2013). Edible insects are commonly used as food in Africa, Latin America or Asia (Raheem et al., 2018). According to the FAO, over 1900 different species of insects are consumed worldwide (FAO, 2013). These include crickets, mealy larvae, ants, grasshoppers and flies to mention a few (van Huis, 2013).

Whole insect meals can be added to food and act as an enriching additive (Duda et al., 2019; Pauter et al., 2018). However, European consumers do not accept eating entire insects, neither larvae nor adults (Hartmann, Shi, Giusto, & Siegrist, 2015). Therefore, one of the methods to increase their acceptability and consumption is the supply of insects in milled form. Some countries from European Union have regulated the production of insect-based foods. A global change in legislation in this area may contribute to the increase of insect consumption in Europe (C/2017/8878).

Crickets (*Acheta domesticus*) are extremely valuable in terms of

nutritional value. They do not contain only significant amounts protein or fat, but also of vitamins and minerals (Ayieko, Ogola, & Ayieko, 2016; Stull et al., 2018). However, depending on the species of insect, its development stage, type of breeding (insect farm or caught in the wild), the protein content varies considerably, from 13 to over 77% of dry matter (Kouřimská & Adámková, 2016). Ecological and environmental conditions, processing of insects including type of heat treatment (boiling, baking, roasting) also significantly affect the nutritional value of edible insects (de Castro, Ohara, dos Santos Aguilar, & Domingues, 2018). The available data suggests that consumption of insects may have positive impact on human health. Stull et al. (2018) state that it may stimulate the growth of intestinal microbiota and decrease the level of TNF- α in plasma. Moreover, chitin and chitosan, substances abundant in crickets, were found to suppress pathogenic microorganisms in the intestines (Liu et al., 2010). Most of the research, however, was conducted on laboratory-grown crickets. However, there is inadequate of data concerning commercially available processed cricket powders.

Therefore, the aim of this study was to investigate the nutrient composition, including fat, mineral, protein and peptide contents in selected edible cricket powders of different origins available on European market in order to verify their quality.

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2. Materials and methods

2.1. Samples

Three edible cricket powders (roasted and finely milled adult *Acheta domesticus*) were investigated. The powders (CP1, CP2 and CP3) were obtained from three commercial suppliers. The products were advertised as supplements or additives rich in protein and were available on the English market. They were purchased at online shops. The crickets were raised in various non-European Union countries (the country of origin of CP1 and CP2: Thailand, CP3: Canada).

2.2. Chemicals

Acetonitrile (LC-MS grade) was purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Formic acid (MS grade) was obtained from Sigma-Aldrich (Schnelldorf, Germany). Lyophilized sequence grade modified trypsin (Gold) was obtained from Promega GmbH (Mannheim, Germany). All other chemicals were purchased from Sigma-Aldrich, in their best available purity grade.

2.3. Nutritional value

Determination of the dry matter was carried out in accordance to the AOAC 930.15 method. The total nitrogen content was determined by the Kjeldahl method according to ISO 1871 and was used to calculate the protein content by multiplying the result by the conversion factor of 4.76 (Janssen, Vincken, van den Broek, Fogliano, & Lakemond, 2017). The ash content was determined according to ISO 936:1998, the total fat content – according to AACC 30-25.01, the total water content – according to ISO 1442. The content of dietary fibre, both soluble (SDF) and insoluble (IDF), were determined by the enzymatic method in accordance to the AACC method 32-07.01. The carbohydrate content was estimated by subtracting the average content of ash, fat, fibre and protein from 100%. The energy value (EV) [kcal/100 g] was calculated using the following formula (Manzi, Aguzzi, & Pizzoferrato, 2001):

$$\text{EV} = 4 \times \text{protein}(\%) + 4 \times \text{carbohydrate}(\%) + 2 \times \text{fibre}(\%) + 9 \times \text{lipid}(\%).$$

2.4. Minerals content

The concentrations of minerals – Ca, Cu, Fe, K, Mg, Mn, Na and Zn – were determined using flame atomic absorption spectroscopy (F-AAS) (SpectraAA-800, Varian, USA) preceded by mineralization with nitric acid (Rybicka & Gliszczynska-Swiglo, 2017). The percentages of Recommended Dietary Allowances (RDA) and Adequate Intakes (AI) were calculated according to the recommendations of the European Parliament and of the Council (European Parliament and Council, 2011).

2.5. Protein profile

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed to compare protein profiles of cricket powders and to estimate the extent of protein aggregation and degradation. A sample of 10 mg was solubilized with lysis buffer (8 M urea, 2 M thiourea, 0.05 mM Tris, 75 mM dithiothreitol (DTT), 3% SDS, 0.05% bromophenol blue, at pH = 6.8) and heated at 98 °C for 4 min. Protein (15 µL) aliquots were loaded onto 15% polyacrylamide gels prepared in a Hoefer SE250 system (GE Healthcare Bio-Sciences). Broad range molecular weight standard (Bio-Rad) was applied. Gels were run at a constant current of 20 mA per gel, then stained with Coomassie brilliant blue and scanned (ImageMaster scanner, GE Healthcare Bio-Sciences). The powders were analysed in duplicate.

2.6. Trypsin digestion

CP samples (0.3 g) were homogenised with 100 mM of aqueous ammonium bicarbonate, using a T25 Ultra-Turrax (IKA Labortechnik, Staufen, Germany) at 9500 rpm for 2 × 20 s, followed by 13,500 rpm for 30 s. Homogenized suspensions were dried under vacuum using a miVac Duo Concentrator (Genevac Ltd., Suffolk, UK). Dry samples (10 mg) were rehydrated in 100 µL of 50 mM ammonium bicarbonate. The proteins were reduced with 200 mM dithiothreitol (56 °C for 1 h) and then alkylated using 200 mM iodoacetamide (30 min in the dark at room temperature). The remaining iodoacetamide was quenched by the addition of 200 mM DTT and incubation at room temperature for 30 min. The samples were digested in an ammonium bicarbonate solution containing 0.083 µg/µL trypsin, at 37 °C, overnight. The digests were purified by reversed-phase extraction using Sep-Pak C18 Plus cartridges (sorbent weight 360 mg/0.7 mL; Waters, Milford, MA, USA). Eluted peptides were dried under vacuum. For chromatographic separation, samples were resuspended in 2% acetonitrile in milli-Q water containing 0.1% formic acid before analysis by UHPLC-Q-TOF-MS/MS.

2.7. Protein and peptide identification

UHPLC-QTOF-MS/MS analysis was performed with the use of an Agilent Technologies (Santa Clara, CA, USA) 1290 Infinity series liquid chromatograph composed of a binary pump, a thermostat and an autosampler, coupled to a 6550 UHD iFunnel Q-TOF LC/MS. Compounds were ionised by electrospray ionisation (ESI) using a JettStreamTechnology ion source. Chromatographic separation was performed on a 2.1 × 150 mm, 1.8 µm particle size Agilent RRHD Eclipse Plus C18 column. Instrument control and data acquisition were performed using Agilent MassHunter Workstation Software. The LC parameters were set as follows: 10 µL injection volume, 0.3 mL/min mobile phase flow. The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). Gradient steps were applied as follows: 0–2 min, 3% B; 2–40 min, to 35% B; 40–45 min, to 40% B; 45–50 min, to 90% B; 50–55 min, 90% B, and a 5 min post-run at 3% B. The ion source gas (nitrogen) temperature was 250 °C and the flow rate was 14 L/min, nebulizer pressure was 35 psi, sheath gas temperature was 250 °C, sheath gas flow was 11 L/min. The capillary voltage was set at 3500 V. Nozzle voltage was set at 1000 V and the fragmentor voltage at 400 V. Positive ions were acquired in the range of 100–3000 *m/z* in MS scan mode and in auto MS/MS mode, with a scan rate of 9 scan/s for MS and 7 scan/s for MS/MS. Internal mass calibration was enabled, two reference ions of *m/z* 121.0509 and 922.0098 were used.

A UniProtKB/Swiss-Prot database search for proteins and peptides identification was performed, using the Spectrum Mill MS Proteomics Workbench (Agilent Technologies) with precursor isolation purity > 70% and 10 ppm precursor mass tolerance, with the following parameters: trypsin enzyme, taxonomy all, two missed cleavages, 50 ppm products mass tolerance, carbamidomethylation as fixed modification, methionine oxidation as a variable modification, and 3+ maximum ambiguous precursor charge. MS/MS search results were validated using Spectrum Mill auto thresholds strategy and peptide mode, which automatically optimizes the score and R1-R2 score thresholds until they reach a specified target FDR. The target FDR was set to 1.2%, which results in an actual FDR of 1%. The list of identified proteins and peptides was reported. Selected peptides, in FASTA format, were searched against the NCBI database for species and protein specificity using the Protein BLAST Alignment Search Tool and blastp algorithm (USA National Library of Medicine).

2.8. Statistical analysis

All measurements were repeated in triplicate unless stated otherwise. One-way analysis of variance (ANOVA) was carried out

Table 1
Nutritional value of cricket powders.

Sample	Protein [%]	Fat [%]	Ash [%]	Fibre [%]		Carbohydrate ¹ [%]	Energy value ²	
				IDF	SDF		[kcal/100 g]	[kJ/100 g]
CP1	45.8 ± 0.6 ^a	23.7 ± 0.3 ^b	4.3 ± 0.2 ^a	5.7 ± 0.1 ^b	0.9 ± 0.1 ^a	19.6 ± 0.5 ^c	488.1 ^b	2042.2 ^b
CP2	42.0 ± 0.4 ^c	29.1 ± 0.6 ^a	3.6 ± 0.3 ^b	2.9 ± 0.2 ^c	0.6 ± 0.1 ^b	21.8 ± 0.8 ^a	524.1 ^a	2192.8 ^a
CP3	44.8 ± 0.5 ^b	23.6 ± 0.5 ^b	4.2 ± 0.3 ^{ab}	6.4 ± 0.1 ^a	1.0 ± 0.2 ^a	20.0 ± 0.7 ^b	486.4 ^b	2035.1 ^b

Mean values in columns denoted by different letters differ statistically significantly ($p < 0.05$).

¹ The carbohydrate content was estimated by subtracting the average content of ash, fat, fibre and protein from 100%.

² Energy value was calculated based on average protein, fat and carbohydrate content.

Table 2
Mineral composition of CPs.

Sample	Ca	K	Mg	Na	Cu	Fe	Mn	Zn
RDA/AI [mg]	1000	4700	320	1500	0.9	18	1.8	8
Content [mg/100 g]								
CP1	218 ± 4 ^a	1224 ± 68 ^a	113 ± 2 ^a	312 ± 17 ^a	4.51 ± 0.06 ^a	4.06 ± 0.20 ^b	4.10 0.17 ^c	13.5 ± 0.4 ^b
CP2	139 ± 9 ^c	826 ± 32 ^c	110 ± 4 ^a	284 ± 18 ^a	2.33 ± 0.05 ^b	4.07 ± 0.30 ^b	12.5 ± 0.6 ^a	21.8 ± 0.7 ^a
CP3	163 ± 6 ^b	993 ± 27 ^b	86 ± 4 ^b	263 ± 16 ^a	4.30 ± 0.29 ^a	5.99 ± 0.07 ^a	7.25 ± 0.78 ^b	12.8 ± 0.9 ^b
% RDA/AI								
CP1	22%	26%	35%	21%	501%	23%	228%	169%
CP2	14%	18%	34%	19%	259%	23%	694%	273%
CP3	16%	21%	27%	18%	478%	33%	403%	160%

Mean values in columns denoted by different letters differ statistically significantly ($p < 0.05$).

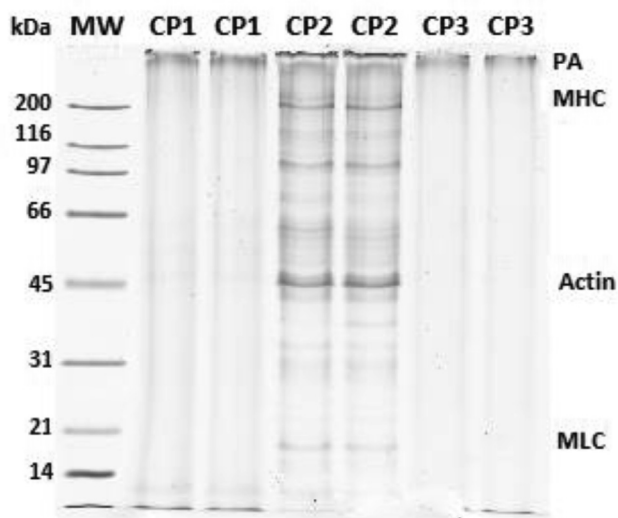


Fig. 1. SDS-PAGE of protein profile of edible cricket powders. Abbreviations denote bands of protein aggregates (PA), myosin heavy chains (MHC), myosin light chains (MLC).

independently for each dependent variable. Post-hoc Tukey HSD multiple comparison test was used to identify statistically homogeneous subsets at $\alpha = 0.05$. Statistical analysis was performed with Statistica 13 software (Dell Software Inc., USA).

3. Results and discussion

3.1. Nutritional value of cricket powders

Nutritional composition of selected cricket powders is presented in Table 1. All analysed CPs were rich in protein which was their dominant constituent. Protein content ranged from 42.0% for CP2 to 45.8% for CP1. Proximate composition of crickets depends on its origin, stage of life, sex and feed (de Castro, Ohara, dos Santos Aguilar, & Domingues, 2018). This results in diversified nutritional value of CPs.

Insect proteins are known for their high nutritional value attributed to high digestibility and desirable amino acid profile (Rumpold & Schlüter, 2013). The fat content in the analysed samples ranged from 23.6% for CP3 to 29.1% for CP2 which made fat the second predominant of CP. Kim et al. (2016) reported that palmitic acid, oleic acid and linoleic acid were the major fatty acids in crickets products. Noteworthy, the highest fat content is present in crickets at the larval stage of life (Tzompa-Sosa, Yi, Van Valenberg, Van Boekel, & Lakemond, 2014). CPs also differed in the content of dietary fibre, both SDF and IDF. In this context, CP3 was found to contain the highest amount of dietary fibre (6.4% and 1.0% IDF and SDF, respectively), while was found to contain the least CP2 (2.9% and 0.6% IDF and SDF, respectively). Literature review indicates the comparable content of dietary fibre (about 4%) in crickets (Rumpold & Schlüter, 2013; Zielińska, Baraniak, Karaś, Rybczyńska, & Jakubczyk, 2015). The content of carbohydrates ranged between 19.6 and 21.8%. The energy content in the analysed CPs ranged from 486 kcal for CP3 to 524 kcal/100 g for CP2 (2035 to 2193 kJ/100 g, respectively).

The content of minerals in edible insects depends on numerous factors, eg. seasonal and feeding conditions (Manditsera, Luning, Fogliano, & Lakemond, 2019) and therefore some differences were found for CPs. From all minerals, the contents of Ca, K, Mg, Na, Cu, Fe, Mn and Zn in the analysed cricket powders. Moreover, the percentages of Recommended Dietary Allowance (RDA) and Adequate Intake (AI) were calculated for each product and were also presented in Table 2.

The most frequent mineral deficiencies refer to shortcomings of Ca, Fe and Mg. Therefore, the contents of Ca, Mg and Fe can be considered decisive for the definition of the nutritional value of a product in the aspect of its mineral composition. Although the content of Ca was different in the tested cricket powders, all of them were a rich source of this macroelement. The highest content of Ca (218 mg/100 g) was found in CP1, whereas the lowest (139 mg/100 g) in CP2. Our results were comparable to those obtained by Ghosh and co-authors for *Gryllus bimaculatus* (240 mg/100 g) (Ghosh, Lee, Jung, & Meyer-Rochow, 2017). Field crickets (*Gryllus*) together with *Protetia* (259 mg/100 g) and *Teleogryllus* (193 mg/100 g) were found to be the richest sources of Ca from among edible insects, including *Allomyrina* (123 mg/100 g), *Tenebrio* (78 mg/100 g) (Ghosh, Lee, Jung, & Meyer-Rochow, 2017), *Eulepida* (55–98 mg/100 g) and *Henicus* (49–158 mg/100 g) (Manditsera,

Table 3
Most abundant proteins identified in cricket powders.

Identified protein	Species	Accession no.	Matched peptides ^a	Sequence coverage (%) ^b	Unique score ^c	Total intensity
CP1						
Myosin heavy chain, muscle	DROME (<i>Drosophila melanogaster</i> , fruit fly)	P05661	129	25.7	965.14	1.00E+09
Actin, muscle-type A2	BOMMO (<i>Bombyx mori</i> , domestic silkworm)	P07837	53	67.0	388.6	5.40E+08
Calcium-transporting ATPase sarcoplasmic/endoplasmic reticulum type	DROME (<i>Drosophila melanogaster</i> , fruit fly)	P22700	29	19.7	283.51	1.48E+08
ATP synthase subunit beta, mitochondrial	DROME (<i>Drosophila melanogaster</i> , fruit fly)	Q05825	15	38.2	265.48	2.44E+07
Major capsid protein	Invertebrate iridescent virus 6 (IIV-6) host = " <i>Acheta domesticus</i> (house cricket)"	Q05815	24	46.0	226.59	1.54E+08
Tropomyosin	LOCM1 (<i>Locusta migratoria</i> , migratory locust)	P31816	14	36.7	187.28	1.59E+07
Tubulin alpha chain	ONCMY (<i>Oncorhynchus mykiss</i> , related sequence to <i>Drosophila melanogaster</i>)	P18288	16	32.4	163.7	1.90E+07
ATP synthase subunit alpha, mitochondrial	DROME(<i>Drosophila melanogaster</i> , fruit fly)	P35381	18	19.3	147.53	1.06E+07
Alpha-actinin, sarcomeric	ANOGA (<i>Anopheles gambiae</i> , African malaria mosquito)	Q7PKQ5	12	10.7	144.7	4.25E+06
Tubulin beta-1 chain	MANSE (<i>Manduca sexta</i> , tobacco hornworm)	O17449	16	28.6	140.74	7.45E+06
Arginine kinase	CARMA (<i>Carcinus maenas</i> , green crab)	Q9U9J4	16	18.7	130.57	3.92E+07
Tropomyosin-2	DROME(<i>Drosophila melanogaster</i> , fruit fly)	P09491	11	14.4	88.3	1.99E+07
Troponin T	PERAM (<i>Periplaneta Americana</i> , American cockroach)	Q9XZ71	7	11.1	78.49	7.82E+06
Paramyosin, long form	DROME (<i>Drosophila melanogaster</i> , fruit fly)	P35415	7	6.5	75.02	3.43E+07
Elongation factor 1-alpha	YARLI (<i>Yarrowia lipolytica</i> CLIB122)	O59949	7	13.4	73.03	8.36E+05
CP2						
Myosin heavy chain, muscle	DROME (<i>Drosophila melanogaster</i> , fruit fly)	P05661	108	25.3	1009.52	1.36E+09
Actin-87E	DROME (<i>Drosophila melanogaster</i> , fruit fly)	P10981	42	64.6	380.49	2.53E+07
Calcium-transporting ATPase sarcoplasmic/endoplasmic reticulum type	ANOGA (<i>Anopheles gambiae</i> , African malaria mosquito)	Q7PPA5	28	21.6	354.73	2.41E+08
Alpha-actinin, sarcomeric	ANOGA (<i>Anopheles gambiae</i> , African malaria mosquito)	Q7PKQ5	17	20.7	284.33	9.52E+06
Tubulin beta-1 chain	MANSE (<i>Manduca sexta</i> , tobacco hornworm)	O17449	23	48.3	249.47	2.02E+07
ATP synthase subunit beta, mitochondrial	DROME (<i>Drosophila melanogaster</i> , fruit fly)	Q05825	14	39.0	243.12	3.88E+07
Tubulin alpha-1 chain	DROME (<i>Drosophila melanogaster</i> , fruit fly)	P06603	12	45.5	222.25	3.88E+07
Tropomyosin	LOCM1 (<i>Locusta migratoria</i> , migratory locust)	P31816	13	36.7	210.87	1.49E+07
ATP synthase subunit alpha, mitochondrial	DROME (<i>Drosophila melanogaster</i> , fruit fly)	P35381	12	23.0	176.11	1.51E+07
Arginine kinase	CARMA (<i>Carcinus maenas</i> , green crab)	Q9U9J4	9	18.7	129.73	2.46E+07
Tropomyosin-1	BOMMO (<i>Bombyx mori</i> , domestic silkworm)	Q1HPU0	10	22.1	116.14	7.45E+07
ADP, ATP carrier protein	DROME (<i>Drosophila melanogaster</i> , fruit fly)	Q26365	6	18.5	73.06	2.22E+07
Troponin T	PERAM (<i>Periplaneta Americana</i> , American cockroach)	Q9XZ71	7	9.8	84.78	2.29E+07
Elongation factor 2	DROME (<i>Drosophila melanogaster</i> , fruit fly)	P13060	7	8.6	73.06	4.51E+06
14-3-3 protein zeta	BOMMO (<i>Bombyx mori</i> , domestic silkworm)	Q2F637	5	21.0	72.46	9.83E+06
CP3						
Myosin heavy chain, muscle	DROME (<i>Drosophila melanogaster</i> , fruit fly)	P05661	110	25.1	960.59	6.62E+08
Actin-87E	DROME (<i>Drosophila melanogaster</i> , fruit fly)	P10981	48	65.9	362.59	1.51E+06
Calcium-transporting ATPase sarcoplasmic/endoplasmic reticulum type	DROPS (<i>Drosophila pseudoobscura</i> , fruit fly)	Q292Q0	26	17.5	267.96	9.29E+06
ATP synthase subunit beta, mitochondrial	DROME (<i>Drosophila melanogaster</i> , fruit fly)	Q05825	16	34.4	215.4	1.77E+07
Tropomyosin	LOCM1 (<i>Locusta migratoria</i> , migratory locust)	P31816	11	33.5	174.47	8.26E+06
Alpha-actinin, sarcomeric	ANOGA (<i>Anopheles gambiae</i> , African malaria mosquito)	Q7PKQ5	7	8.7	122.24	2.60E+06
Arginine kinase	CARMA (<i>Carcinus maenas</i> , green crab)	Q9U9J4	15	18.6	109.64	4.51E+07
Tubulin beta-1 chain	MANSE (<i>Manduca sexta</i> , tobacco hornworm)	O17449	9	22.1	93.64	3.10E+06

(continued on next page)

Table 3 (continued)

Identified protein	Species	Accession no.	Matched peptides ^a	Sequence coverage (%) ^b	Unique score ^c	Total intensity
Tropomyosin-2	DROME (<i>Drosophila melanogaster</i> , fruit fly)	P09491	8	14.4	87.21	1.66E+07
Tubulin alpha-1 chain	DROME (<i>Drosophila melanogaster</i> , fruit fly)	P06603	8	15.3	81.15	8.64E+06
ATP synthase subunit alpha, mitochondrial	DROME (<i>Drosophila melanogaster</i> , fruit fly)	P35381	7	12.3	78.88	1.12E+07
Paramyosin, long form	DROME (<i>Drosophila melanogaster</i> , fruit fly)	P35415	5	5.8	78.44	1.78E+07
Troponin T	PERAM (<i>Periplaneta americana</i> , American cockroach)	Q9XZ71	4	7.2	48.99	2.76E+06
Elongation factor 1-alpha	YARLI (<i>Yarrowia lipolytica</i> CLIB122)	O59949	3	9.5	45.27	9.31E+05
Histone H2B	ANOGA (<i>Anopheles gambiae</i> , African malaria mosquito)	Q16P20	3	19.3	36.46	4.15E+06

^a Number of matched peptides in the database search.

^b Percentage of coverage of the entire amino acid sequence.

^c Spectrum Mill unique score, calculated by adding scores of all the unique peptides detected for the protein (scores greater than 15 for Agilent Q-TOF instrument represent valid results).

Luning, Fogliano, & Lakemond, 2019). The content of Mg in all powders found within the range of 86 to 113 mg/100 g, whereas that of Fe ranged between 4.06 and 5.99 mg per 100 g. These contents correspond to 27–35% of RDA and 23–33% of RDA for Mg and Fe, respectively, that made them a good source of Mg and Fe. However, other insects like *Eulepida* (Mg: 226–298 mg/100 g, Fe: 25–54 mg/100 g) (Manditsera, Luning, Fogliano, & Lakemond, 2019) or *Protaetia* (Mg: 328 mg/100 g, Fe: 16.2 mg/100 g) (Ghosh, Lee, Jung, & Meyer-Rochow, 2017) were found to be better sources of these minerals.

The content of microelements (Cu, Mn and Zn) in the analyzed cricket powder was extremely high. A portion of 100 g of each powder delivered more than 100% of RDA for all these elements. Among all the insects mentioned in the previous paragraph, crickets were found to be the richest source of Cu, Mn and Zn (Ghosh, Lee, Jung, & Meyer-Rochow, 2017, Manditsera, Luning, Fogliano, & Lakemond, 2019, Nowak, Persijn, Rittenschober, & Charrondiere, 2016). The content of these microelements is of concern as the determined levels were close to the tolerable upper intake level (UL) for a 100 g portion (EFSA, 2018). For example, in CP1 and CP2 Cu was 4.51 mg and 4.30 mg, respectively, whereas the UL for Cu is 5 mg per day. The content of Zn in CP2 (UL of 25 mg per day) was also close to its upper limit of intake. It is worth to point out, however, that cricket powders are used rather as food additives, not final food products. Therefore, the addition of e.g. 5% of cricket powder to any food product would improve its mineral profile while remaining safe.

CP has a potential to become a valuable additive in the production of different products e.g. gluten-free products for people with coeliac disease. This group of consumers is especially vulnerable in terms of proper diet balance including adequate supply of minerals (Rybicka, 2018). Many studies indicate mineral deficiencies among people on a gluten-free diet. Most of them refer to Mg, Fe, Zn, Cu (Vici, Belli, Biondi, & Polzonetti, 2016). According to Rujner et al. (2004), the level of Mg is insufficient in 20% of patients on gluten-free diets. Fasano and Catassi (2001) state that, because of Fe malabsorption, anemia accompanies coeliac disease in 15% of cases. Moreover, the observations of Halfdanarson, Litzow, & Muttay (2007) and Bottaro, Cataldo, Spina, & Corazza (1999) indicated that anemia caused by Fe deficiency, but not related to malabsorption of this mineral, occurs in up to 50% of people with subclinical stage of coeliac disease. Because of the abundance of both macro- and microelements in CP, its application to gluten-free products could help in the prevention of mineral deficiencies often accompanying coeliac disease.

3.2. Protein and peptide identification

Reliable identification of protein and peptides in food products is challenging because the results are affected by technological operations. Heat treatment during baking or roasting have one of the most destructive effects on proteins in food products. High temperature causes, on the one hand, protein denaturation and aggregation due to changes in the solubility of proteins, on the other hand, it causes substantial protein degradation and breakdown of subunits and chains into shorter fragments. However, heating may positively affect insect proteins at the peptide level, despite the damage of their spatial structure. Edible insects, including crickets, are rich in proteins that were found to be a valuable source of bioactive peptides with antioxidant activity, and their bioactive properties were improved following thermal treatment, e.g. boiling and baking (Zielińska, Baraniak, & Karaś, 2018).

The profiles of proteins extracted from the roasted cricket powders were shown in Fig. 1. The heat treatment applied during production resulted in the appearance of protein aggregates that accumulated on the top of the lanes of all analysed samples. Substantial protein degradation and disappearance of almost all protein bands except for high molecular weight protein aggregates was observed in the case of CP1 and CP3. Based on the result obtained for sample CP2, it seems likely that the conditions of the thermal treatment were less severe during the processing of this powder. The proteins in this sample showed less degradation as multiple bands were visible in the range between 200 kDa and 10 kDa. Thermal degradation of insect proteins was observed previously, when electrophoretic patterns of proteins extracted from raw, boiled and baked mealworm, locust and cricket samples were compared (Zielińska, Baraniak, & Karaś, 2017). Moreover, the extent of damage of the proteins in meat was found to be mainly related to the type of technological operation and the conditions of processing (Di Luccia et al., 2015; Tornberg, 2005). Actin, which is one of the most thermostable proteins of meat, turned out to be completely degraded in the examined cricket powders (samples CP1 and CP3). This indicates that both samples were processed under very harsh conditions. In our previous study, actin bands were partially preserved even in sterilised meat products while all other proteins were degraded (Montowska & Fornal, 2017).

Noteworthy, heat treatment may have a positive effect on the microbial safety of insect based products (Grabowski & Klein, 2016) and insects have been found to be a feedstock that may pose some risks because of a significant microbial contamination (Caparros Megido et al., 2017). Moreover, active peptides, a group of bioactive compounds found in crickets, are of a small molecular mass, and, as such, are not as prone to thermal degradation as larger proteins (Agyei,

Table 4
Cricket proteins and selected peptide markers identified in roasted cricket powders.

Identified protein	Accession no.	Matched peptides	Sequence coverage	Protein score	Peptide marker	Parent ion <i>m/z</i>	Mr(calc)	Peptide abundance
CP1								
Translational elongation factor-1 alpha, partial	ACY44265	21	29.6	114.61	EHALLAFTLGVK IGGIGTVPVGR LPLQDVYK TLIEALDAILPPSRPTEKPLR	433.5845 (3+) 513.3100 (2+) 488.2805 (2+) 583.3435 (4+)	1298.7467 1025.6102 975.5510 2330.3442	4.50E+05 1.18E+07 9.83E+06 6.83E+06
Histone 3, partial	ACY74636	7	14.6	21.72	STELLIR YRPGTVALR	416.2512 (2+) 516.8021 (2+)	831.4934 1032.5949	8.58E+06 8.00E+05
Protein kinase, partial	ACY45327	4	7.2	13.91	FALDIAR	403.2335 (2+)	805.4567	5.47E+06
Apolipoprotein-III	AAA64737	4	7.0	7.46	AAELSGDAQTAVR*	644.8389 (2+)	1288.6492	1.24E+07
Glucose phosphate dehydrogenase, partial	ACY46034	2	5.1	6.83	LSNHLNLFK*	586.8309 (2+)	1172.6422	4.72E+06
CP2								
Translational elongation factor-1 alpha, partial	ACY44265	32	43.1	172.25	IGGIGTVPVGR LPLQDVYK MDSTEPPYSEAR TLIEALDAILPPSRPTEKPLR	513.3108 (2+) 488.2810 (2+) 691.7995 (2+) 583.3426 (4+)	1025.6102 975.551 1382.5893 2330.3442	1.88E+07 2.39E+07 1.55E+06 3.45E+06
Apolipoprotein-III	AAA64737	40	66.8	166.97	AAELSGDAQTAVR* QQFPDGAQAADK*	644.8292 (2+) 638.3023 (2+)	1288.6492 1275.5964	3.07E+07 2.27E+06
Histone 3, partial	ACY74636	12	14.7	108.58	VQEAQPHADAVAESLK EIAQDFK	896.4677 (2+) 425.7208 (2+)	1791.9236 850.4305	2.11E+07 1.26E+06
Protein kinase, partial	ACY45327	9	8.5	18.83	STELLIR FALDIAR	416.2519 (2+) 403.2334 (2+)	831.4934 805.4567	1.31E+07 1.27E+07
Glucose phosphate dehydrogenase, partial	ACY46034	3	5.1	6.65	IIPQYHNSR LSNHLNLFK*	620.8419 (2+) 586.831 (2+)	1240.6797 1172.6422	6.17E+06 8.64E+06
CP3								
Translational elongation factor-1 alpha, partial	ACY44265	11	38.3	49.98	EHALLAFTLGVK IGGIGTVPVGR TLIEALDAILPPSRPTEKPLR	433.5889 (3+) 513.3108 (2+) 583.3426 (4+)	1298.7467 1025.6102 2330.3442	5.50E+05 2.26E+06 1.97E+06
Histone 3, partial	ACY74636	9	55.9	25.25	STELLIR YRPGTVALR	416.2513 (2+) 516.8018 (2+)	831.4934 1032.5949	1.02E+06 1.39E+06
Apolipoprotein-III	AAA64737	4	66.8	17.97	AAELSGDAQTAVR*	430.2281 (3+)	1288.6492	4.89E+06
Syntaxin, partial	ACY44581	3	8.2	10.13	QLEITGR	408.7331 (2+)	816.4574	1.19E+06
Protein kinase, partial	ACY45327	4	13.4	7.71	FALDIAR	403.2337 (2+)	805.4567	4.96E+06
Glucose phosphate dehydrogenase, partial	ACY46034	4	5.1	5.28	LSNHLNLFK*	586.831 (2+)	1172.6422	3.22E+06

* Peptide is unique to the genus *Acheta* (field crickets).

yet been sequenced and only partial sequences are available for some of them. With the lack of cricket-specific sequences in the NCBI database, proteins of the most closely related species (e.g., fruit fly, locust or mosquito) were identified. For instance, there are no sequences of cricket myofibrillar proteins available at present in the NCBI database. Therefore, the identified MHC peptide sequences were mainly associated to the second part of the MHC isoforms derived from fruit fly, with over a hundred peptides detected (Table 3). The comparison of the fruit fly and locust MHC isoforms is shown in Fig. 2. Identified peptides are highlighted. Previously, identification of edible insects, i.e. buffalo worms, mealworms, crickets and grasshoppers, purchased on the market, was performed based on generation of specific fingerprint mass spectra of a given insect (Ulrich, Kühn, Biermaier, Piacenza, Schwaiger, Gottschalk, & Gareis, 2017).

Interestingly, detection of major capsid protein derived from invertebrate iridescent virus, also called Chilo iridescent virus (Jakob, Müller, Bahr, & Darai, 2001), may indicate that the batch of crickets used for manufacture was infected with this virus. Iridovirus infections of herbivore insects, with symptoms ranging from mild to very severe, have particular economic and ecological impact on agriculture. The major capsid protein, identified in this study with high score and intensity (Table 3), represents around half of the total protein mass of the virion (Jakob, Müller, Bahr, & Darai, 2001, Stohwasser, Raab, Schnitzler, Janssen, & Darai, 1993).

The final stage was to identify peptide markers specific to processed house cricket protein. For this purpose, all 206 house cricket (*Acheta domestica*) protein entries present in the NCBI database were imported into the Spectrum Mill MS Proteomics Workbench. The entries consisted mainly of cytoplasmic proteins. Many of them were only partial. In general, the same proteins were identified in the examined samples, such as translational elongation factor-1 alpha, apolipophorin-III, histone 3, protein kinase, glucose phosphate dehydrogenase (Table 4). Protein BLAST was used to search NCBI database for species and protein specificity of the peptides identified in the samples. Table 4 present the selected heat-stable peptides detected in cricket powders. The peptide abundance ranged from 10^5 - 10^7 . Included in these are four cricket-specific peptides (unique exclusively to the genus *Acheta*) identified with respect to the current state of knowledge expressed in the NCBI database. Three of these peptides were unique to cricket apolipophorin-III (AAELSGDAQTAVR, QQFPDGAQAADK and VQEAV-QPHADAVAESLK) and one – to glucose phosphate dehydrogenase (LSNHLNLFK).

4. Conclusions

Crickets have been consumed for a long time, especially in Africa, Latin America and Asia. Cricket powders (CPs), obtained from adult crickets (*Acheta domestica*), contain significant amounts of nutritionally valuable constituents. They contained a significant amount of protein (55–60%), fat (24–29%), fibre (3.5%–7%) and minerals. The content of analysed minerals in 100 g of the products was: Ca (139–218 mg), K (826–1224 mg), Mg (86–113 mg), Na (263–312 mg), Cu (2.33–4.51 mg), Fe (4.06–5.99 mg), Mn (4.1–12.5 mg) and Zn (12.8–21.8 mg). Therefore, they may become a valuable food ingredients for different food products. The high mineral content of CP could make it an additive in products targeted at people suffering from coeliac disease.

Moreover, investigations into the proteins of CP with SDS-PAGE revealed that the processing of the raw material caused complete protein degradation in two out of three samples. This suggests that the powders were subjected to harsh thermal treatment.

Finally, proteins of CP were identified using mass spectrometry. Four cricket-specific peptides were found in the trypsin lysates of CP that could serve as markers for authentication purposes.

Conflict of interest

The authors declare that they have no conflict of interest for publishing this research.

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References

- Aggei, D., Ongkudon, C. M., Yi Wei, C., Chan, A. S., & Danquah, M. K. (2016). Bioprocess challenges to the isolation and purification of bioactive peptides. *Food and Bioprocess Processing*, 98, 244–256. <https://doi.org/10.1016/j.fbp.2016.02.003>.
- Ayieko, M. A., Ogola, H. J., & Ayieko, I. A. (2016). Introducing rearing crickets (gryllids) at household levels: Adoption, processing and nutritional values. *Journal of Insects as Food and Feed*, 2(3), 203–211. <https://doi.org/10.3920/JIFF2015.0080>.
- Bottaro, G., Cataldo, F., Spina, M., & Corazza, G. R. (1999). The clinical pattern of sub-clinical/silent celiac disease: an analysis on 1026 consecutive cases. *American Journal of Gastroenterology*, 94, 691–696. <https://doi.org/10.1111/j.1572-0241.1999.00938.x>.
- C/2017/8878. Commission implementing regulation (EU) 2017/2470 of 20 December 2017 establishing the Union list of novel foods in accordance with Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods.
- Caparros Megido, R., Desmedt, S., Blecker, C., Béra, F., Haubruge, É., Alabi, T., & Francis, F. (2017). Microbiological load of edible insects found in Belgium. *Insects*, 8(1), 12. <https://doi.org/10.3390/insects8010012>.
- De Casto, R. J. S., Ohara, A., dos Santos Aguilar, J. G., & Domingues, M. A. F. (2018). Nutritional, functional and biological properties of insect proteins: Processes for obtaining, consumption and future challenges. *Trends in Food Science & Technology*, 76, 82–89. <https://doi.org/10.1016/j.tifs.2018.04.006>.
- Di Luccia, A., la Gatta, B., Nicastro, A., Petrella, G., Lamacchia, C., & Picariello, G. (2015). Protein modifications in cooked pork products investigated by a proteomic approach. *Food Chemistry*, 172, 447–455. <https://doi.org/10.1016/j.foodchem.2014.09.099>.
- Duda, A., Adamczak, J., Chelmińska, P., Juskiewicz, J., & Kowalczyński, P. (2019). Quality and nutritional/textural properties of durum wheat pasta enriched with cricket powder. *Foods*, 8, 46. <https://doi.org/10.3390/foods8020046>.
- EFSA. (2018). Overview on tolerable upper intake levels as derived by the scientific committee on food (SCF) and the EFSA panel on dietetic products, nutrition and allergies (NDA). Summary of tolerable upper intake levels – version 4 (September 2018). https://www.efsa.europa.eu/sites/default/files/assets/UL_Summary_tables.pdf [visited: 11/11/2018].
- European parliament and council, regulation (EU) No 1169/2011 of 25 October 2011 on the provision of food information to consumers, amending regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European parliament and of the council, and repealing commission directive 87/250/EEC, council directive 90/496/EEC, commission directive 1999/10/EC, directive 2000/13/EC of the European parliament and of the council, commission directives 2002/67/EC and 2008/5/EC and commission regulation (EC) No 608/2004 Text with EEA relevance.
- Fasano, A., & Catassi, C. (2001). Current approaches to diagnosis and treatment of celiac disease: An evolving spectrum. *Gastroenterology*, 120, 636–651. <https://doi.org/10.1053/gast.2001.22123>.
- Food and Agriculture Organization of the United Nations (FAO). (2012). Assessing the potential of insects as food and feed in assuring food security. Presented at Tech. Consult. Meet., 23–25 January, FAO, Rome, Italy.
- Food and Agriculture Organization of the United Nations (FAO). (2013). Edible insects. Future prospects for food and feed security. FAO Forestry Paper 171, FAO, Rome, Italy.
- Ghosh, S., Lee, S. M., Jung, C., & Meyer-Rochow, V. B. (2017). Nutritional composition of five commercial edible insects in South Korea. *Journal of Asia-Pacific Entomology*, 20, 686–694. <https://doi.org/10.1016/j.aspen.2017.04.003>.
- Grabowski, N. T., & Klein, G. (2016). Microbiology of cooked and dried edible Mediterranean field crickets (*Gryllus bimaculatus*) and superworms (*Zophobas atratus*) submitted to four different heating treatments. *Food Science and Technology International*, 23(1), 17–23. <https://doi.org/10.1177/1082013216652994>.
- Halfdanarson, T. R., Litzow, M. R., & Muttay, J. A. (2007). Hematologic manifestations of celiac disease. *Blood*, 109, 412–421. <https://doi.org/10.1182/blood-2006-07-031104>.
- Hartmann, C., Shi, J., Giusto, A., & Siegrist, M. (2015). The psychology of eating insects: A cross-cultural comparison between Germany and China. *Food Quality and Preference*, 44, 148–156. <https://doi.org/10.1016/j.foodqual.2015.04.013>.
- Jakob, N. J., Müller, K., Bahr, U., & Darai, G. (2001). Analysis of the first complete DNA sequence of an invertebrate iridovirus: Coding strategy of the genome of chilo iridescent virus. *Virology*, 286, 182–196. <https://doi.org/10.1006/viro.2001.0963>.
- Janssen, R. H., Vincen, J.-P., van den Broek, L. A. M., Fogliano, V., & Lakemond, C. M. M. (2017). Nitrogen-to-protein conversion factors for three edible insects: *Tenebrio molitor*, *Alphitobius diaperinus*, and *Hermetia illucens*. *Journal of Agricultural and Food Chemistry*, 65(11), 2275–2278. <https://doi.org/10.1021/acs.jafc.7b00471>.
- Kim, D.-H., Kim, E.-M., Chang, Y.-J., Ahn, M.-Y., Lee, Y.-H., Park, J. J., & Lim, J.-H. (2016). Determination of the shelf life of cricket powder and effects of storage on its quality characteristics. *Korean Journal of Food Preservation*, 23(2), 211–217 <https://doi.org/10.1100/kjfp.2016.23.2.211>.

- doi.org/10.11002/kjfp.2016.23.2.211.
- Kouřimská, L., & Adámková, A. (2016). Nutritional and sensory quality of edible insects. *NFS Journal*, 4, 22–26. <https://doi.org/10.1016/j.nfs.2016.07.001>.
- Liu, P., Piao, X. S., Thacker, P. A., Zeng, Z. K., Li, P. F., Wang, D., & Kim, S. W. (2010). Chito-oligosaccharide reduces diarrhea incidence and attenuates the immune response of weaned pigs challenged with *Escherichia coli* K881. *Journal of Animal Science*, 88, 3871–3879.
- Manditsera, F. A., Luning, P. A., Fogliano, V., & Lakemond, C. M. M. (2019). The contribution of wild harvested edible insects (*Eulepida mashona* and *Henicus whellani*) to nutrition security in Zimbabwe. *Journal of Food Composition and Analysis*, 75, 17–25. <https://doi.org/10.1016/j.jfca.2018.09.013>.
- Manzi, P., Aguzzi, A., & Pizzoferrato, L. (2001). Nutritional value of mushrooms widely consumed in Italy. *Food Chemistry*, 73(3), 321–325. [https://doi.org/10.1016/S0308-8146\(00\)00304-6](https://doi.org/10.1016/S0308-8146(00)00304-6).
- Montowska, M., & Fornal, E. (2017). Label-free quantification of meat proteins for evaluation of species composition of processed meat products. *Food Chemistry*, 237, 1092–1100. <https://doi.org/10.1016/j.foodchem.2017.06.059>.
- Montowska, M., & Pospiech, E. (2016). Processed meat protein and heat-stable peptide marker identification using microwave-assisted tryptic digestion. *Food Technology and Biotechnology*, 54, 482–488. <https://doi.org/10.17113/ftb.54.04.16.4540>.
- Nowak, V., Persijn, D., Rittenschober, D., & Charrondiere, U. R. (2016). Review of food composition data for edible insects. *Food Chemistry*, 193, 39–46. <https://doi.org/10.1016/j.foodchem.2014.10.114>.
- Pauter, P., Róžańska, M., Wiza, P., Dworczak, S., Grobelna, N., Sarbak, P., & Kowalczewski, P. L. (2018). Effects of the replacement of wheat flour with cricket powder on the characteristics of muffins. *Acta Scientiarum Polonorum - Technologia Alimentaria*, 17(3), 227–233. <https://doi.org/10.17306/J.AFS.2018.0570>.
- Raheem, D., Carrascosa, C., Oluwole, O. B., Nieuwland, M., Saraiya, A., Millan, R., & Raposo, A. (2018). Traditional consumption of and rearing edible insects in Africa, Asia and Europe. *Critical Reviews in Food Science and Nutrition*, 58.
- Rujner, J., Socha, J., Syczewska, M., Wojtasik, A., Kunachowicz, H., & Stolarczyk, A. (2004). Magnesium status in children and adolescents with coeliac disease without malabsorption symptoms. *Clinical Nutrition*, 23, 1074–1079. <https://doi.org/10.1016/j.clnu.2003.10.018>.
- Rumpold, B. A., & Schlüter, O. K. (2013). Nutritional composition and safety aspects of edible insects. *Molecular Nutrition & Food Research*, 57(5), 802–823.
- Rybicka, I. (2018). The handbook of minerals on a gluten-free diet. *Nutrients*, 10(11), 1683. <https://doi.org/10.3390/nu10111683>.
- Rybicka, I., & Gliszczyńska-Świgło, A. (2017). Minerals in grain gluten-free products. The content of calcium, potassium, magnesium, sodium, copper, iron, manganese, and zinc. *Journal of Food Composition and Analysis*, 59, 61–67. <https://doi.org/10.1016/j.jfca.2017.02.006>.
- Stohwasser, R., Raab, K., Schnitzler, P., Janssen, W., & Darai, G. (1993). Identification of the gene encoding the major capsid protein of insect iridescent virus type 6 by polymerase chain reaction. *Journal of General Virology*, 74, 873–879.
- Stull, V. J., Finer, E., Bergmans, R. S., Febvre, H. P., Longhurst, C., Manter, D. K., ... Weir, T. L. (2018). Impact of edible cricket consumption on gut microbiota in healthy adults, a double-blind, randomized crossover trial. *Scientific Reports*, 8, 10762. <https://doi.org/10.1038/s41598-018-29032-2>.
- Tornberg, E. (2005). Effects of heat on meat proteins - implications on structure and quality of meat products. *Meat Science*, 70, 493–508. <https://doi.org/10.1016/j.meatsci.2004.11.021>.
- Tzompa-Sosa, D. A., Yi, L. Y., Van Valenberg, H. J. F., Van Boekel, M. A. J. S., & Lakemond, C. M. M. (2014). Insect lipid profile: Aqueous versus organic solvent-based extraction methods. *Food Research International*, 62, 1087–1094.
- Ulrich, S., Kühn, U., Biermaier, B., Piacenza, N., Schwaiger, K., Gottschalk, C., & Gareis, M. (2017). Direct identification of edible insects by MALDI-TOF mass spectrometry. *Food Control*, 76, 96–101. <https://doi.org/10.1016/j.foodcont.2017.01.010>.
- van Huis, A. (2013). Potential of insects as food and feed in assuring food security. *Annual Review of Entomology*, 58, 563–583. <https://doi.org/10.1146/annurev-ento-120811-153704>.
- Vici, G., Belli, L., Biondi, M., & Polzonetti, V. (2016). Gluten free diet and nutrient deficiencies: A review. *Clinical Nutrition*, 35, 1236–1241. <https://doi.org/10.1016/j.jand.2018.04.007>.
- Zielińska, E., Baraniak, B., & Karaś, M. (2017). Antioxidant and anti-inflammatory activities of hydrolysates and peptide fractions obtained by enzymatic hydrolysis of selected heat-treated edible insects. *Nutrients*, 9, 970. <https://doi.org/10.3390/nu9090970>.
- Zielińska, E., Baraniak, B., & Karaś, M. (2018). Identification of antioxidant and anti-inflammatory peptides obtained by simulated gastrointestinal digestion of three edible insects species (*Grylodes sigillatus*, *Tenebrio molitor*, *Schistocerca gregaria*). *International Journal of Food Science and Technology*, 53, 2542–2551. <https://doi.org/10.1111/ijfs.13848>.
- Zielińska, E., Baraniak, B., Karaś, M., Rybczyńska, K., & Jakubczyk, A. (2015). Selected species of edible insects as a source of nutrient composition. *Food Research International*, 77, 460–466. <https://doi.org/10.1016/j.foodres.2015.09.008>.