



Original article

Characterising cricket flour from *Gryllus assimilis*: an alternative source of nutrients for sustainability

Ezequiel Toribio,¹ María Jimena Correa,¹  Sandra Karina Medici,² Cristina Ferrero¹ & Carlos Gabriel Arp^{1*} 

¹ Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), Facultad de Ciencias Exactas-Universidad Nacional de La Plata, Comisión de Investigaciones Científicas de la Provincia de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas, 47 y 116, La Plata CP 1900, Argentina

² Instituto de Investigaciones en Producción, Sanidad y Ambiente (IIPROSAM), CONICET-UNMDP, Centro de Asociación Simple Comisión de Investigaciones Científicas, Funes 3350, Mar del Plata CP 7600, Argentina

(Received 4 June 2024; Accepted in revised form 19 August 2024)

Summary A trend to study entomological options as food is turning the practice of entomophagy into a novel research field in search for sustainable and accessible sources of nutrients. In this work, cricket (*Gryllus assimilis*) was processed into flour (CF), and then extracted using different solvents to obtain oils and defatted flours. The repeatability of CF obtention, its nutritional profile, and some of its physicochemical properties were studied. Results showed repeatability with a 28% mass yield for CF production. The CF presented adequate amino acid, mineral, and fatty acid profiles, a high content of dietary fibre, and low available carbohydrates, thus being considered a suitable alternative for inclusion in healthy human diets. Using data obtained from the total nitrogen content, the amino acid profile, and the total dietary fibre determinations, the CF protein digestibility was estimated at 67.4%. Based on experimental data, a mean nitrogen-to-protein conversion factor of 5.76 was calculated for a *G. assimilis* meal. The study showed that nutritional cricket flour and derivatives can be obtained using non-sophisticated methods.

Keywords amino acid score, cricket flour, fatty acid composition, mineral profile, nitrogen-to-protein conversion factor, techno-functional properties.

Introduction

Entomophagy, the human consumption of insects, is an ancestral tradition in several cultures and is gaining global attention as a sustainable food practice. The FAO's 2013 publication 'Edible insects: future prospects for food and feed security' highlighted the economic, environmental, and nutritional advantages of insects in modern diets, spreading worldwide interest on this topic and the need for regulatory research (van Huis *et al.*, 2013).

More than 2100 insect species are considered edible (Jongema, 2017), but only a small number of species, such as *Locusta migratoria*, *Acheta domesticus*, and *Tenebrio molitor*, has been thoroughly researched and approved in Western countries, especially in the European Union (Pasini *et al.*, 2022). Despite cultural biases, the demand for insect-based ingredients is growing due to their high nutritional value and sustainability. Insects offer a rich source of proteins and

essential amino acids, with a caloric profile favouring lipids over carbohydrates, making them ideal for healthier, low-carbohydrate diets.

Moreover, entomophagy is key to achieving global sustainability goals due to its efficient use of resources (González *et al.*, 2019). Insect farming has a lower environmental footprint compared with traditional livestock, requiring less land, water, and feed, and emitting fewer greenhouse gases (Finke, 2002; Rumpold & Schlüter, 2013). Insects also provide an efficient tool for contributing to the circular economy since they can degrade food waste, thus reducing and repurposing biological materials.

This study focuses on *Gryllus assimilis*, a cricket species gaining interest in Argentina and South America, which was processed into flour using traditional methods. The research aims to characterise the nutritional quality of cricket flour and its derivatives, including defatted flour and extracted oils. Additionally, a method to discriminate the nitrogen compounds as a tool to estimate the nitrogen-to-protein conversion factor was proposed.

*Correspondent: Fax: 54-221-4254853; e-mail: carp@exactas.unlp.edu.ar

Materials and methods

Materials

Crickets (*G. assimilis*) were provided by the farm Grillos Capos (Argentina). The specimens were 40–50 days old, and were cultured, reared, and harvested under standardised conditions according to the recommendations of the European Food Safety Authority (EFSA). All chemicals were of analytical-grade.

Cricket flour production

The specimens were selected and separated from the substrate (composed of food remains and excreta) and then weighed. Then, they were scalded in boiling water for 180 s and drained. The specimens were distributed homogeneously in oven plates avoiding the overlap of the individual specimens and were dehydrated at 130 °C for 60 min in a convection oven (Lanín II, Panier, Argentina). Finally, dried samples were ground and stored in an airtight plastic container until further analysis. This product was identified as cricket flour (CF).

Characterisation

The obtained CF was characterised in terms of composition by total nitrogen, fat, moisture, ash, and total dietary fibre (TDF) by the corresponding approved AACC methods 46-12.01, 30-25.01, 44-15.02, 08-01.01, and 32-05.01 (AACC, 2000) (available carbohydrate content determined by difference). Additionally, amino acid profile of proteins was obtained by high performance liquid chromatography (HPLC), mineral composition was determined by inductively coupled plasma optical emission spectrometer (ICP-OES) and ICP mass spectrometer (ICP-MS), and fatty acids profile by gas chromatography (GC) after methylation. Chitin was calculated based on experimental data. On the other hand, CF was subjected to partial defatting with different solvents, and the following techno-functional properties of the whole and the defatted flours were determined: water-holding capacity (WHC), oil absorption capacity (OAC), parameters (L^* , a^* , b^*) with a surface colorimeter; as well as the fatty acid composition of both the defatted flours and the extracted oils. An exhaustive description of all procedures and methods used for the characterisation of the CF, the partially defatted flours, and the oils are supplied in Appendix S1.

Results and discussion

Cricket flour production: Yield and stability

The dehydration conditions used for the production of the CF were effective in reducing the water from the

specimens. The process showed good repeatability and an overall mass yield of 28 ± 1 (% w/w, $n = 4$), with the majority of the mass loss corresponding to water (initial water content: 79.0 ± 0.8 g H₂O/100 g crickets, $n = 9$). The final water content of the ground sample was 1.28 ± 0.1 g H₂O/100 g CF (wb). The final product had an a_w of 0.185 at 20 °C that guarantees microbiological stability during storage in adequate conditions.

Low values of a_w could increase lipid oxidative susceptibility, especially the autooxidation of unsaturated fatty acids. However, there is a minimum fat oxidation activity in the range of 0.18–0.32 a_w values, so this phenomenon would be limited (Bonazzi & Dumoulin, 2011). Counterintuitively, some authors have reported that heating processes performed on mealworms (*T. molitor*), crickets (*A. domesticus*), and locusts (*Schistocerca gregaria* and *L. migratoria*) led to enhanced oxidative stability in comparison to other methods, especially freeze-drying, likely due to the formation of Maillard products during heating (Kröncke et al., 2018; Lenaerts et al., 2018; Keil et al., 2022).

Proximate composition of cricket flour and nutritional analysis

Energy (471 kJ) and proximate composition of the CF are listed in Table S1. The major constituents were crude proteins (61.2%) and fats (24.5%). The rest was distributed into TDF (8.3%), ashes (4.5%), and available carbohydrates (1.5%). These data are consistent with data for the Gryllidae family reported elsewhere (Rumpold & Schlüter, 2013; Soares Araújo et al., 2019).

Minerals

The CF mineral profile was collected to further investigate the presence of macro, micro, and oligo-elements (Ca, K, Mg, Na, P, Cu, Fe, Mn, Zn, Co, Mo, Se, I) as well as some heavy metals and other minerals (Al, Cr, Ni, Pb, Cd, As) potentially harmful to consumers (Table 1).

It was found that the K concentration of the CF was higher than the highest value found in the literature (1500 mg/100 g, Rumpold & Schlüter, 2013). Although there is wide variability in the mineral contents among studies, all the other measured minerals and elements were in the ranges reported by several authors for the Gryllidae family (Rumpold & Schlüter, 2013; Zielińska et al., 2015; Soares Araújo et al., 2019; Kosečková et al., 2022).

From the recommended dietary allowances (RDA) and the adequate intakes (AdI) published elsewhere (Oria et al., 2019), consuming 25 g of dry CF would contribute significantly to the RDA of P, Cu, Fe, Zn, Mo, and Se; and the AdI of Mn.

No As or Cd was detected in CF, but some concentrations of Al, Cr, Ni, and Pb were, so adopting

Table 1 Mineral profile of CF, recommended mineral intakes, and CF's mineral contribution

	CF ^a	RDA or Adl ^b	CF mineral contribution ^c
Ca	68 ± 2	1000–1300	1.3–1.7
K	1914 ± 88	2600–3400*	14.1–18.4
Mg	91.10 ± 0.02	310–400	5.7–7.4
Na	415.0 ± 0.1	1500*	6.9
P	880 ± 14	700	31.5
Cu	1.71 ± 0.05	0.9	47.4
Fe	7.0 ± 0.3	8–18	9.7–21.8
Mn	2.23 ± 0.09	1.6–2.3*	24.2–34.8
Zn	15.2 ± 0.9	8–11	34.6–47.6
Al	1.56 ± 0.00	—	—
Cr	0.08 ± 0.01	0.020–0.035*	54.3–95.0
Ni	0.06 ± 0.01	—	—
Pb	0.09 ± 0.01	—	—
Cd	nd	—	—
Co	nd	—	—
Mo	0.058	0.045	32.1
Se	0.026	0.055	12.0
I	nd	—	—
As	nd	—	—

nd, not detected.

^aCricket flour, in mg/100 g, dry basis.

^bRecommended dietary allowances or adequate intakes (values followed by *): males and females (19–65 years old), in mg. From Oria et al. (2019).

^c%RDA or Adl covered by 25 g (dry) of CF.

policies regarding these hazardous chemicals is recommended when producing ingredients derived from insects. However, the concentration of Pb found in CF (0.09 mg/100 g = 0.009 ppm) was lower than the different limits established by the MERCOSUR Guidelines for cereals and cereal flours, animal foods, and edible ice (MERCOSUR, 2011).

Nitrogen compounds

Proteins, amino acids, and chitin. The analysis of nitrogen compounds in insect samples is important due to the significant presence of non-protein nitrogen compounds, particularly chitin. The total nitrogen (Nt) measured by the Kjeldahl method was 9.80 g N/100 g CF (db). In most food samples, using the Nt value and a nitrogen-to-protein conversion factor would give a crude protein value close to the actual protein content due to the negligible presence of non-protein nitrogen compounds. For whole insect samples, using the Nt value for calculating the protein content would lead to undesirable overestimations (Jonas-Levi & Martinez, 2017).

A better approach to more accurate protein and protein nitrogen (Np) values can be achieved using the amino acid profile by HPLC (Table 2). According to the results, the protein content of the CF based on the

Table 2 Indispensable amino acids profile of cricket flour

	CF ^a	CF protein ^b	FAO (2007) ^b	AAS ^c	ePDCAAS ^d
Phe + Tyr	5.96	121.62	22	5.53	3.73
Phe	4.79	97.67	—	—	—
Tyr	1.18	24.06	—	—	—
Hys	ND	ND	15	ND	ND
Ile	2.07	42.23	30	1.41	0.95
Leu	5.23	106.66	59	1.81	1.22
Lys	3.49	71.22	45	1.58	1.07
Met + Cys	2.83	57.22	22	2.62	1.77
Met	1.21	24.67	16	1.54	1.04
Cys	1.62	33.03	6	5.51	3.72
Thr	1.14	23.24	23	1.01	0.68
Val	1.71	34.77	39	0.89	0.60
Trp	0.91	18.47	6	3.08	2.08

ND, Non-detected.

^aExpressed as g amino acid per 100 g CF (% w/w), dry basis.

^bExpressed as mg amino acid per g CF protein, dry basis.

^cAmino acid score.

^dEstimated protein digestibility corrected amino acid score (digestibility factor: 0.674).

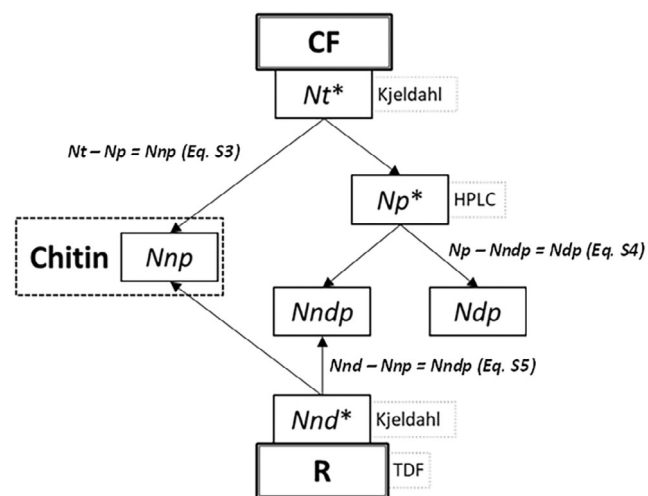


Figure 1 Nitrogen analysis from CF and the indigestible residue (R) from the TDF method. The * symbol indicates experimental values. Ndp, digestible protein nitrogen; Nnd, non-digestible protein; Nndp, non-digestible protein nitrogen; Nnp, non-protein nitrogen; Np, protein nitrogen; Nt, total nitrogen. eqn S3–S5 are displayed in Appendix S1.

amino acid count is up to 49.0 g/100 g CF (db), with this value corresponding to 7.48 g N/100 g CF (db), which is the protein nitrogen (Np). Then, given the value of Nt obtained by Kjeldahl (9.80 g N/100 g CF, db), the remaining 2.32 g N/100 g CF (db) would correspond to non-protein nitrogen (Nnp), which is assumed to be mainly chitin (Fig. 1).

Chitin is a polysaccharide composed of 2-acetamido-2-deoxy-d-glucopyranose and 2-amino-2-deoxy-d-

glucopyranose that can be found in nature with a variable acetylation degree (Simionatto Guinesi & Gomes Cavalheiro, 2006). This polymer can be found mainly bonded with proteins, being part of the arthropod's exoskeleton cuticle (Muthukrishnan *et al.*, 2019). The amount of this polysaccharide in the sample can be calculated through the *Nnp*. However, given that the ratio between the N mass in chitin and its molecular mass depends on the acetylation degree (AD) of the polymer, only a range of chitin concentration can be calculated without an accurate knowledge of the chitin's AD in each particular sample. Assuming that chitin is defined as a $0.5 < AD < 1.0$ polymer, typically 0.9 (Pillai *et al.*, 2009; Zargar *et al.*, 2015), the range of chitin concentration in the CF is estimated to be 2.97–3.31 g chitin/100 g CF (db).

In vitro protein availability. Analysing the sources of nitrogen in the residues from the TDF determination can provide even more information (Fig. 1). Assuming that TDF residues are mostly composed by chitin and proteins associated with it, that is, the non-digestible fraction of the proteins (Manditsera *et al.*, 2019), their nitrogen content would be the sum of the non-protein nitrogen (*Nnp*) and the non-digestible protein nitrogen (*Nndp*). Furthermore, given that chitin is considered an insoluble fibre (Zargar *et al.*, 2015; Kim *et al.*, 2023), it is possible to assume that $Nnp \approx$ nitrogen from chitin. Then, the *Nndp* and the nitrogen from the digestible protein fraction (*Ndp*) can be calculated by means of the eqn S3–S5.

Then, given the values of *Nt* and *Nnd* obtained by the Kjeldahl method (9.80 and 4.76 g N/100 g CF (db), respectively), the value of *Np* obtained by HPLC (7.48 g N/100 g CF (db)), and the calculated *Nnp* (2.32 g N/100 g CF (db), eqn S3), the values of *Ndp* and *Nndp* were 5.04 and 2.44 g N/100 g CF (db), respectively (eqn S4 and S5). This result suggests, through eqn S6, that 67.4% of the protein nitrogen would be available for absorption after the digestion, given an approximate value of protein digestibility. This result is in agreement with Turck *et al.* (2021), who informed a protein digestibility of 67.3% for *A. domesticus*.

Analysis of the amino acids profile and nitrogen-to-protein conversion factor. The proteins' nutritional quality and nitrogen-to-protein conversion factors depend on the amount and proportion of their amino acids. The indispensable amino acids profile of the CF is shown in Table 2, while the complete amino acid profile is shown in Table S2.

The proteins from CF are rich in aromatic amino acids, such as phenylalanine, tyrosine, and tryptophan (ePDCAAS ≥ 1). Moreover, they also contain high concentration of sulfurous amino acids, especially

cysteine. Leucine and lysine are also adequately present in CF proteins. Conversely, histidine was identified as the limiting amino acid since it was not detected. Proteins from CF are also relatively low in isoleucine, and very low in threonine and valine.

For comparison, and based on the amino acid profiles from different varieties of wheat cultivars (Han *et al.*, 2019; Siddiqi *et al.*, 2020), it was found that CF proteins had similar contents of minor amino acids, namely valine and threonine. However, CF proteins would be an adequate complement for increasing the lysine and methionine contents of such cereal.

On the other hand, the data from the complete amino acid profile can be introduced into eqn S8 and S9 to obtain the conversion factors, which in this case yielded values of $F_{Nt \rightarrow P}$, that is, the nitrogen-to-protein conversion factor, of 4.97 and $F_{Np \rightarrow P}$, that is, the pure protein conversion factor, of 6.54. These values were similar to those reported elsewhere for different insect species, although there is a wide range of variability depending on the study ($F_{Nt \rightarrow P}$ varies from 2.9 to 5.33, and $F_{Np \rightarrow P}$ from 5.49 to 6.0 for the Orthoptera order) (Janssen *et al.*, 2017; Mishyna *et al.*, 2019; Boulos *et al.*, 2020; Ritvanen *et al.*, 2020). The difference between the two values is explained by the *Nnp* fraction that in this case is as high as the 23.7% of the total N in the sample. Mosse (1990) proposed that the average value between $F_{Nt \rightarrow P}$ and $F_{Np \rightarrow P}$ would give a more accurate general N-protein conversion factor ($F_{N \rightarrow P}$) since they represent the lower and upper limits for the experimental conversion factors, respectively. In the case of the present study, the calculated value for $F_{N \rightarrow P}$ was 5.76. Thus, using this proposed conversion factor, the actual protein content for CF was 56.4%.

Available carbohydrates

It is worth noting the low content of digestible carbohydrates in CF, since the main carbohydrate is chitin, a nitrogen polysaccharide with recognised benefits for health that is generally assumed to be indigestible so it would be included in the TDF. On the other hand, the amount of carbohydrates not comprised in the TDF is speculated to be part of the food remaining in the gastrointestinal tract of the specimens (Finke, 2002).

Fatty acid profile

Lipids supply energy and provide essential fatty acids, impacting human health. Moreover, the lipid composition impacts food design since it modifies food palatability and can influence in the shelf life of products. Table S3 presents the fatty acid profiles of CF and partially defatted cricket flours. CF's main fatty acids were linoleic (34.7%), oleic (27.7%), and palmitic (25.9%) acids, which led to a polyunsaturated fatty acids (PUFA)-to-saturated fatty acids (SFA) ratio,

Σ PUFA/ Σ SFA \sim 1, and to a ω -6/ ω -3 ratio \sim 40, the latter being far from the value generally recognised as recommendable. However, these ratios are being questioned because they are too general and the effect on health of all SFA or unsaturated fatty acids (UFA) is not the same. For instance, it is generally considered that all PUFA reduce the low-density lipoprotein cholesterol (LDL-C) and that the opposite effect is caused by SFA. However, a 'raising effect' has been proposed as follows for SFA: myristic > lauric > palmitic > medium-chain fatty acids > stearic (Calder, 2015). Thus, other indices have been proposed that consider the role of fatty acids, for instance, in lipid metabolism, coagulation, and the risk of cardiovascular disease (Chen & Liu, 2020). Among these indices are the atherogenic index (AI), the thrombogenic index (TI), the hypocholesterolemic/hypercholesterolemic index (HH), and the nutritive value index (NVI).

The AI for CF was 0.43 which is considered a low value. Food products with low AI could contribute to reducing the levels of serum cholesterol. Values of AI ranging from 0.084 to 0.55 have been reported for crops and 1.42–5.13 for dairy products (Chen & Liu, 2020). Regarding edible insects, *A. domesticus* adult and larval forms presented values of 0.42 and 0.46, respectively (Orkusz, 2021), similar to those reported in this study for adults of *G. assimilis*.

The TI of CF was low (0.93). TI provides a relationship between the pro-thrombogenic fatty acids (SFA) and the anti-thrombogenic ones (UFA) so higher TI values are related to a greater contribution to clot formation in vessels. Finally, the HH index reflects the relationship between the fatty acids considered hypocholesterolemic (oleic acid and PUFA) and the ones considered hypercholesterolemic (SFA). Thus, higher HH values are associated with a protective action against cardiovascular disease. The HH value for CF was 2.38 which is higher than the reported values for lamb meat (1.92–2.01) and dairy products (0.32–1.29) (Chen & Liu, 2020).

The oxidative stability is relevant in ingredients with high lipid content and with a high level of UFA, such as the CF. For this reason, the Cox parameter that shows the tendency to oxidation was evaluated. Samples with higher Cox values are more prone to oxidation and are less stable. The Cox of CF was high, which reflected its rich content of UFA.

Organic solvent extraction: Lipid analysis of defatted flours and oils

One-step CF extraction with ethanol, petroleum ether, or hexane led to partially defatted CF (PDCF-E, PDCF-P, and PDCF-H, respectively) and impacted the fatty acid profile of defatted flours and oils in different manners. Petroleum ether and hexane

showed a similar and higher capacity for extraction than that of ethanol (extraction yields of 52.7%, 55.1%, and 37.0% for petroleum ether, hexane, and ethanol, respectively).

The AI of PDCF-E was slightly but significantly increased with respect to CF, showing that UFA was extracted to a greater extent than SFA, while a slight reduction of AI was observed when CF was defatted with hexane and petroleum ether. However, in all cases, the AI values are considered low.

The TI of CF and defatted flours was low in all cases (0.81–0.99) but the flours obtained using hexane and petroleum ether presented significantly lower values. Finally, the HH values of PDCF-P and PDCF-H were slightly but significantly higher than CF and PDCF-E, showing an improved fatty acid profile.

Regarding oxidative stability, the Cox values were between 3.81 (PDCF-E) and 5.84 (PDCF-H), which reflected the higher content of SFA of PDCF-E and the higher level of UFA in PDCF-H. Although these values are high, other factors such as the presence of antioxidants and the handling conditions can modify this predisposition to oxidation. In addition, in the partially defatted flours (PDCF) this process could be less relevant due to the lower lipid content (\sim 9.1–13.5%).

The lipid profile of extracted fats and the derived indices is shown in Table S4. The UFA were the predominant, being the linoleic acid the principal, followed by the oleic and palmitic acids. The AI and the TI of all the samples were low enough, and the HH high enough, to predict a positive effect on health.

Finally, the high Cox values predict short-medium oxidation stability. This susceptibility to oxidation can be improved by the action of antioxidants and adequate storage conditions.

Organic solvent extraction: CF and defatted flours techno-functional properties

The WHC and OAC are parameters relevant to predicting the effects on food formulations.

Cricket flour presented a WHC significantly different from all PDCF (Fig. 2a). The extraction with ethanol led to a slight reduction of WHC while extraction with petroleum ether and hexane led to a slight increase. Mokaya *et al.* (2024) extracted proteins using polar solvents (water or alkaline solution) on hexane-defatted flours from *Gryllus bimaculatus*, *Gynanisa maja*, and *Gonimbrasia belina*. The authors found a significantly higher WHC value for the *G. bimaculatus* protein extract as the efficiency of the protein extraction was also higher, likely due to the higher protein concentration of this sample, and stated that

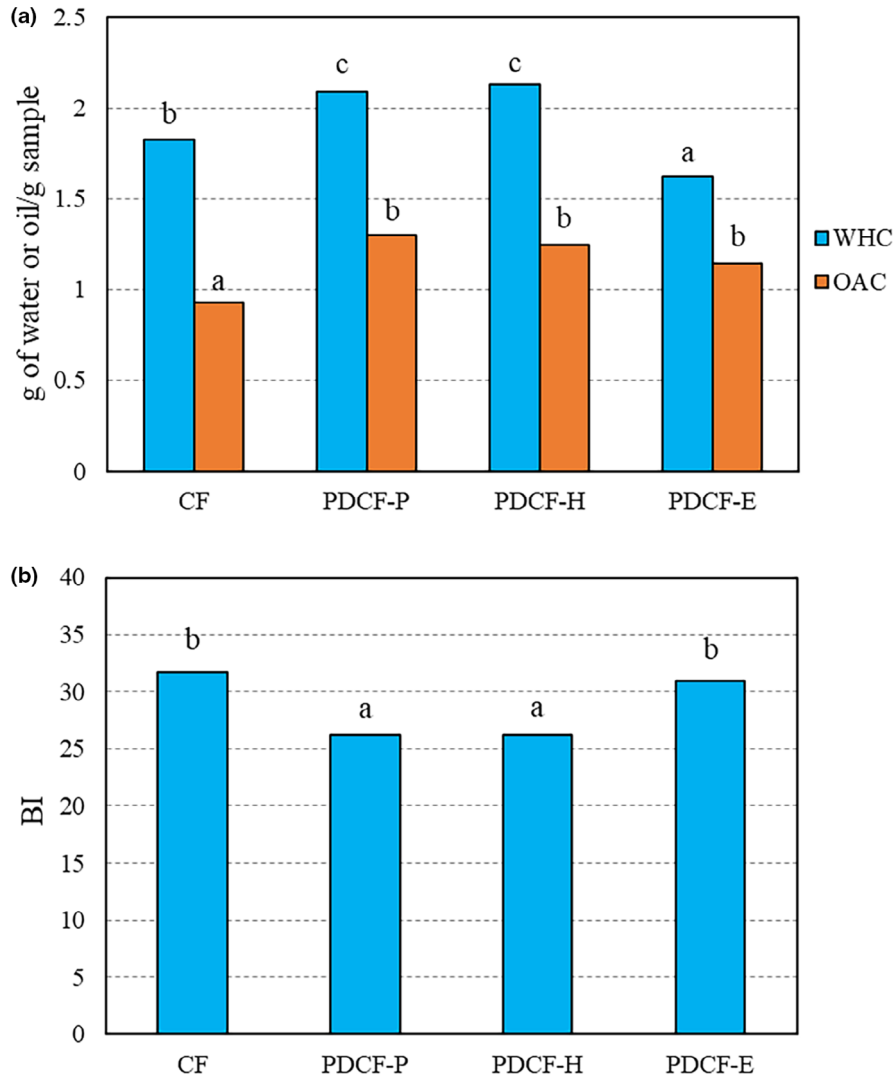


Figure 2 Techno-functional properties of CF and partially defatted flours. (a) water-holding capacity (WHC) and oil absorption capacity (OAC); (b) browning index (BI). Different letters in the same group of bars indicate significant differences ($P < 0.05$).

remaining lipids can block the access of water to hydrophilic parts of the proteins. In the case of CF, defatting with ethanol led to the lowest extraction efficiency of lipids, thus resulting in a PDCF with proportionally less protein and more lipids that led to a lower WHC value. The opposite would be valid for PDCF-H and PDCF-P, which presented lipid extraction efficiencies higher than 50%.

The OAC is a parameter that is related to the contribution of an ingredient to food palatability, mouth feel, and flavour retention. As expected, the PDCF presented a higher ability to retain oil than CF, which was reflected in higher values of OAC for all the PDCF (Fig. 2a), as the hydrophobic sites of the CF proteins (composed of 45%, 42%, and 13% of

hydrophobic, hydrophilic, and amphipathic amino acids, respectively) would be more accessible in the PDCF to interact with oil after the endogenous lipids were extracted.

Figure 2b shows the colour indices calculated from the CIE $L^*a^*b^*$ colour space. The browning index (BI), which is related to the brownish purity, was slightly modified by ethanol extraction (PDCF-E) with respect to CF, but considerably reduced in PDCF-H and PDCF-P. This behaviour was also reflected in the values of ΔE^* , which were significantly higher for PDCF-H and PDCF-P (15.7 ± 1.3 and 16.7 ± 0.6 , respectively) in comparison to PDCF-E (2.6 ± 0.8). The results suggest that samples defatted with hexane and petroleum ether would be perceived as having a different colour in comparison to CF

by the human eye since ΔE^* was higher than 3, while the one defatted with ethanol would be perceived as the same colour as CF ($\Delta E^* < 3$) (Martínez-Cervera *et al.*, 2012).

Conclusions

The chemical evaluation of cricket flour (CF) from *G. assimilis* and its derivatives demonstrated the high nutritional value of the samples, characterised by abundant proteins and lipids. The amino acid profile of CF showed an adequate amount of essential amino acids, and allowed us to calculate a conversion factor of nitrogen content to protein mass for *G. assimilis* flour for the first time. Moreover, an experimental design combining amino acid profile, total nitrogen, and TDF was proposed for discriminating nitrogen fractions and estimating protein digestibility.

This study demonstrated that lipids in CF have beneficial nutritional properties, and that the techno-functional properties of CF can be modified by a one-step defatting process. Entomophagy, the practice of eating insects, is gaining popularity in Western countries so any efforts to investigate such products are beneficial to its widespread adoption.

Conflict of interests

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Acknowledgments

The authors would like to thank Lic. Caporaletti from Grillos Capos for providing the specimens and counselling for their manipulation, and Mg. Lenz and Dra. Gallardo from Instituto Nacional de Tecnología Industrial (INTI) for the counselling for the cricket flour production. The research was done with funds of the Universidad Nacional de La Plata (project UNLP X661 and X771) and the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT, PICT 2019-2593, PICT-2020-SERIEA-02634 and PICT 2019-00877). The funding sources had no involvement in the preparation of this article.

Ethics approval was not required for this research.

Author contributions

Ezequiel Toribio: Investigation; formal analysis; data curation. **María Jimena Correa:** Funding acquisition; writing – original draft; writing – review and editing; visualization; formal analysis; project administration; data curation; supervision; resources. **Sandra Karina Medici:** Investigation. **Cristina Ferrero:** Funding acquisition; writing – review and editing; project

administration; resources. **Carlos Gabriel Arp:** Conceptualization; investigation; funding acquisition; writing – original draft; writing – review and editing; visualization; methodology; formal analysis; project administration; data curation; supervision; resources.

Peer review

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ijfs.17503>.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

References

- Bonazzi, C. & Dumoulin, E. (2011). Quality changes in food materials as influenced by drying processes. In: *Modern Drying Technology: Vol. 3: Product* (edited by E. Tsotsas & A.S. Mujumdar). Pp. 1–20, 1st edn. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co.
- Boulos, S., Tännler, A. & Nyström, L. (2020). Nitrogen-to-protein conversion factors for edible insects on the Swiss market: *T. molitor*, *A. domesticus*, and *L. migratoria*. *Frontiers in Nutrition*, **7**, 1–12.
- Calder, P.C. (2015). Functional roles of fatty acids and their effects on human health. *Journal of Parenteral and Enteral Nutrition*, **39** (1S), 18S–32S.
- Chen, J. & Liu, H. (2020). Nutritional indices for assessing fatty acids: a mini-review. *International Journal of Molecular Sciences*, **21**, 5695.
- Finke, M.D. (2002). Complete nutrient composition of commercially raised invertebrates used as food for insectivores. *Zoo Biology*, **21**, 269–285.
- González, C.M., Garzón, R. & Rosell, C.M. (2019). Insects as ingredients for bakery goods. A comparison study of *H. illucens*, *A. domestica* and *T. molitor* flours. *Innovative Food Science & Emerging Technologies*, **51**, 205–210.
- Han, F., Han, F., Wang, Y. *et al.* (2019). Digestible indispensable amino acid scores of nine cooked cereal grains. *British Journal of Nutrition*, **121**, 30–41.
- van Huis, A., Itterbeek, J.V., Klunder, H. *et al.* (2013). Edible insects: future prospects for food and feed security. In: *FAO Forestry Paper 171*. Rome, Food and Agriculture Organization of the United Nations, Rome and Wageningen University and Research Centre, the Netherlands. 187 pp.
- Janssen, R.H., Vincken, J.-P.P., van den Broek, L.A.M.M., Fogliano, V. & Lakemond, C.M.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**, 2275–2278.
- Jonas-Levi, A. & Martinez, J.J.I. (2017). The high level of protein content reported in insects for food and feed is overestimated. *Journal of Food Composition and Analysis*, **62**(June), 184–188. This commentary article highlights an important issue that we addressed in our work concerning the reported protein values from insects found in literature. Jonas-Levi & Martinez (2017) stated that these values come mostly from total nitrogen quantification, followed by a conversion to protein values using the general 6.25 factor. This strategy does not take into account the nitrogen contribution from the insect cuticle (which

- contains chitin), leading to an overestimation of the actual protein content of samples.
- Jongema, Y. (2017). *World list of edible insects*. Wageningen, The Netherlands: Wageningen University. Available from: <https://www.wur.nl/en/Research-Results/Chair-groups/Plant-Sciences/Laboratory-of-Entomology/Edible-insects/Worldwide-species-list.htm>
- Keil, C., Grebenteuch, S., Kröncke, N. et al. (2022). Systematic studies on the antioxidant capacity and volatile compound profile of yellow mealworm larvae (*T. molitor* L.) under different drying regimes. *Insects*, **13**, 166.
- Kim, D.-H., Wang, Y., Jung, H. et al. (2023). A type 2 immune circuit in the stomach controls mammalian adaptation to dietary chitin. *Science*, **381**, 1092–1098.
- Kosečková, P., Zvěřina, O., Pěchová, M., Krulíková, M., Duborská, E. & Borkovcová, M. (2022). Mineral profile of cricket powders, some edible insect species and their implication for gastronomy. *Journal of Food Composition and Analysis*, **107**, 104340.
- Kröncke, N., Bösch, V., Woyzichowski, J., Demtröder, S. & Benning, R. (2018). Comparison of suitable drying processes for mealworms (*Tenebrio molitor*). *Innovative Food Science and Emerging Technologies*, **50**, 20–25.
- Lenaerts, S., Van Der Borgh, M., Callens, A. & Van Campenhout, L. (2018). Suitability of microwave drying for mealworms (*Tenebrio molitor*) as alternative to freeze drying: impact on nutritional quality and colour. *Food Chemistry*, **254**, 129–136.
- The work of Lenaerts et al. (2018) was one of the first to demonstrate that using heat treatments (e.g., microwave) to dehydrate insect samples results in better oxidative stability compared to freeze-drying, also pointing it out as a not expected result. Different authors (e.g., Kröncke et al., 2018; and Keil et al., 2022) have arrived to similar conclusions on this topic using different heat-based methods (e.g., rack oven, fluidized bed, vacuum oven dry, among others). Therefore, these methods are considered more suitable for producing oxidatively stable dry insect products than freeze-drying.
- Manditsera, F.A., Luning, P.A., Fogliano, V. & Lakemond, C.M.M. (2019). Effect of domestic cooking methods on protein digestibility and mineral bioaccessibility of wild harvested adult edible insects. *Food Research International*, **121**, 404–411.
- Martínez-Cervera, S., Sanz, T., Salvador, A. & Fiszman, S.M. (2012). Rheological, textural and sensorial properties of low-sucrose muffins reformulated with sucralose/polydextrose. *LWT - Food Science and Technology*, **45**, 213–220.
- MERCOSUR. (2011). Reglamento Técnico MERCOSUR sobre límites máximos de contaminantes inorgánicos en alimentos (DEROGACIÓN DE LAS RES. GMC N° 102/94 y N° 35/96). In *MERCOSUR/GMC/RES. N° 12/11*. Available from: http://www.puntofocal.gov.ar/doc/r_gmc_12-11.pdf
- Mishyna, M., Martínez, J.J.I., Chen, J. & Benjamin, O. (2019). Extraction, characterization and functional properties of soluble proteins from edible grasshopper (*Schistocerca gregaria*) and honey bee (*Apis mellifera*). *Food Research International*, **116**(September 2018), 697–706.
- Mokaya, H.O., Mudalungu, C.M., Tchouassi, D.P. & Tanga, C.M. (2024). Techno-functional and antioxidant properties of extracted protein from edible insects. *ACS Food Science & Technology*, **4**, 1130–1141.
- Mosse, J. (1990). Nitrogen to protein conversion factor for ten cereals and six legumes or oilseeds. A reappraisal of its definition and determination. Variation according to species and to seed protein content. *Journal of Agricultural and Food Chemistry*, **38**, 18–24.
- Muthukrishnan, S., Merzendorfer, H., Arakane, Y. & Yang, Q. (2019). Chitin organizing and modifying enzymes and proteins involved in remodeling of the insect cuticle. In: *Targeting Chitin-Containing Organisms* (edited by Q. Yang & T. Fukamizo). Pp. 83–114. Singapore: Springer.
- Oria, M., Harrison, M. & Stallings, V.A. (2019). Dietary reference intakes for sodium and potassium. In: *Dietary reference intakes for sodium and potassium* (edited by V.A. Stallings, M. Harrison & M. Oria). Washington, DC: National Academies Press. <https://doi.org/10.17226/25353>
- Orkusz, A. (2021). Edible insects versus meat—nutritional comparison: knowledge of their composition is the key to good health. *Nutrients*, **13**, 1207.
- Pasini, G., Cullere, M., Vegro, M., Simonato, B. & Dalle Zotte, A. (2022). Potentiality of protein fractions from the house cricket (*Acheta domestica*) and yellow mealworm (*Tenebrio molitor*) for pasta formulation. *LWT*, **164**(June), 113638.
- Pillai, C.K.S., Paul, W. & Sharma, C.P. (2009). Chitin and chitosan polymers: chemistry, solubility and fiber formation. *Progress in Polymer Science*, **34**, 641–678.
- While knowing the exact degree of acetylation of the actual chitin in the sample would be ideal in terms of accuracy, the analysis of the acetylation degree (e.g., via FT-IR) of a complex sample (such as a whole insect flour) is still complicated due to the interference of lipids and proteins. Additionally, the extraction of chitin for a cleaner characterization typically involves strong acids and alkali that can modify the true acetylation degree in the process. Therefore, the typical degree of acetylation for chitin reported by the authors is of critical importance in this manuscript as it is an essential assumption for the approach we are proposing.
- Ritvanen, T., Pastell, H., Welling, A. & Raatikainen, M. (2020). The nitrogen-to-protein conversion factor of two cricket species – *Acheta domestica* and *Gryllus bimaculatus*. *Agricultural and Food Science*, **29**, 1–5.
- Rumpold, B.A. & Schlüter, O.K. (2013). Nutritional composition and safety aspects of edible insects. *Molecular Nutrition & Food Research*, **57**, 802–823.
- Siddiqi, R.A., Singh, T.P., Rani, M., Sogi, D.S. & Bhat, M.A. (2020). Diversity in grain, flour, amino acid composition, protein profiling, and proportion of Total flour proteins of different wheat cultivars of North India. *Frontiers in Nutrition*, **7**, 141.
- Simonatto Guinesi, L. & Gomes Cavalheiro, É.T. (2006). The use of DSC curves to determine the acetylation degree of chitin/chitosan samples. *Thermochimica Acta*, **444**, 128–133.
- Soares Araújo, R.R., dos Santos Benfca, T.A.R., Ferraz, V.P. & Moreira Santos, E. (2019). Nutritional composition of insects *Gryllus assimilis* and *Zophobas morio*: potential foods harvested in Brazil. *Journal of Food Composition and Analysis*, **76**, 22–26.
- Turck, D., Bohn, T., Castenmiller, J. et al. (2021). Safety of frozen and dried formulations from whole house crickets (*Acheta domestica*) as a novel food pursuant to regulation (EU) 2015/2283. *EFSA Journal*, **19**, e06779.
- The EFSA article focused on *Acheta domestica* is one of the most important and referenced works in this growing field of study. Among all the valuable information that it provides, we have noticed that the authors reported an in vitro estimated total nitrogen digestibility value of 67.3% for *A. domestica*. With our approach, we have reached an estimated total nitrogen digestibility of 51.4%, as well as an estimated protein digestibility of 67.4% (based on values of nitrogen from digestible proteins and nitrogen from total proteins) for *G. assimilis*.
- Zargar, V., Asghari, M. & Dashti, A. (2015). A review on chitin and chitosan polymers: structure, chemistry, solubility, derivatives, and applications. *ChemBioEng Reviews*, **2**, 204–226.
- 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.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Detailed materials and methods.