Food supplements from a Grasshopper: A developmental stage-wise evaluation of amino acid profile, protein and vitamins in *Brachystola magna* (Girard)

Paul Baruk Zamudio-Flores1,*, Juan Manuel Tirado-Gallegos1,2, Miguel Espino-Díaz1, Emilio Ochoa-Reyes1, Francisco Hernández-Centeno1,3, María Hernández-González3, Haydee Yajaira López-De la Peña3, René Salgado-Delgado4, Verónica Graciela García-Cano5, Olalla Sánchez-Ortíz6

1Centro de Investigación en Alimentación y Desarrollo (CIAD), A.C., Unidad Cuauhtémoc. Av. Río Conchos S/N Parque Industrial, Apdo. Postal 781. C.P. 31570. Cd. Cuauhtémoc, Chihuahua, México, 2Facultad de Zootecnia y Ecología, Universidad Autónoma de Chihuahua, Periférico Francisco R. Almada Km. 1, C.P. 31453, Chihuahua, Chihuahua, México, 3Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro 1923. C. P. 2315, Buenavista, Saltillo Coahuila, México, 4Instituto Tecnológico de Zacatepec. Calzada Tecnológico No. 27. C.P. 62780. Zacatepec de Hidalgo, Morelos, México, 5Instituto Tecnológico de Ciudad Cuauhtémoc, Av. Tecnológico No. 137. C.P. 31500. Cd. Cuauhtémoc, Chihuahua, México.

INTRODUCTION

Among the nutritional components, proteins (considering the number and type of amino acids) represent a basic and essential aspect since they provide quality aspects and health in all living organisms, including insects (Jonas-Levi and Martínez, 2017). In addition to this, biochemical substances known with the generic name of vitamin, are a priority for cell biological cycles because they are required (at minimum concentrations) to play important roles in metabolism and in most life cycles (Khosravi-Largani et al., 2018). As a food source, insects are potentially nutritious, since they are considered abundant in proteins, fats, and provide a certain amount of minerals and vitamins (Yi et al., 2013). Within the species of insects, Orthoptera order is one of the most common groups (over 20,000 species) in the world (Löffler and Fartmann, 2017; Monter-Miranda et al., 2018; Zielińska et al., 2018) and these stands out over the other insect species because of their high protein value (Ramos-Elorduy et al., 2012; Monter-Miranda et al., 2018; Zielińska et al., 2018). The most common species of this insect and most important in Mexico are *Melanoplus* spp., *Sphenarium purpurascens*, *Sphenarium mexicanus*, *Taeniopoda*...
**Materials and Methods**

**Materials**
Specimens of *Brachystola magna* (Girard) in stages of nymph 3, nymph 4 and adults were collected from the surroundings of the “Valle de Allende”, Chihuahua (Mexico) in bean crops and pasture. Once collected, the insects were washed with distilled water for the removal of external contaminants and the regurgitated of the insect. To obtain eggs (ootheca), hatcheries were established in plastic containers according to the methodology recently reported by Monter-Miranda et al., (2018). The reagents used for the proximal chemical analysis of proteins were of analytical grade, while those used in the chromatographic analyzes were of HPLC grade and were obtained from Sigma-Aldrich (St. Louis, MO, USA). In order to determine the amino acid profile a high-purity L-amino acid kit was purchased from Sigma-Aldrich (Toluca, Estado de Mexico, Mexico).

**Methods**

**Obtaining Brachystola magna flour**
For the analyzes of proteins content, vitamins and the amino acid profile of the *Brachystola magna* samples in the different stages of development, flour samples were obtained, using the methodology recently reported by Monter-Miranda et al. (2018). Samples were washed and lyophilized at a temperature of -40°C at a vacuum of -133 mbar with a lyophilizer (Labconco 77540-00, MO, USA). Lyophilized samples were milled (mill IKA, model M20, IKA Works, Inc., NC, USA) and sifted (Retsch of 425 µm, number 40, Haan, Alemania) to standardize the particle size. Flour samples were stored in airtight bags (Ziploc®, Johnson y Sons, Inc., Racine, WI, EUA) in a dry and light-free environment for further analysis.

**Protein molecular weight quantification and determination**
Protein quantification of *B. magna* was performed on egg, nymph 3, nymph 4 and adult samples in the form of flours using the official method 928.08 of the Association of Official Agricultural Chemists (AOAC, 2002). Proteins molecular weight determination (soluble proteins aggregates) was performed by polyacrylamide gel electrophoresis with sodium dodecyl sulfate (SDS-PAGE) according to the methodology recently reported by Mishyna et al., (2018) with slight modifications. Samples were dissolved in a 0.2 M Tris buffer and incubated for 2 h at 40 °C. Subsequently, they were centrifuged (3,000 × g/30 min), an aliquot was taken mixed with the sample buffer and incubated at 65 °C for 10 min. Samples were evaluated using a 12% gel (Tris-Glycine) with a standard marker in the range of 10-250 kDa.
Amino acid profile

Amino acid profile was determined according to the methodology reported by González Paramás et al., (2006). Amino acids were quantified using a Varian chromatographic system, which consisted of a 9012Q pump, a 9100 auto-injector and a 9075 fluorescent detector. The samples (1.0 g in dry basis, for each of the stages of insect development) were submitted to an automatic precolumn reaction using 100 μL of derivatizing reagent. The chromatographic conditions were as follows: Flow 0.1 mL/min until minute 3 and then 1.5 mL/min; solvents, A, sodium phosphate buffer (10 mM, pH 7.3):methanol: tetrahydrofurane (80:19:1) and B, sodium phosphate buffer (10 mM, pH 7.3):methanol (20:80). The gradient consists of: 100% A during 3.5 min, 0-15% of B in A for 6 min, 15% B isocratically for 5 min, 15-30% of B for 5 min, 30-40% of B for 4 min, and 40-80% of B for 12 min. Separation was performed on a C18 Waters Nova-Pack reverse phase column (particle size 4 μm, 150 × 3.9 mm internal diameter). A specific column guard Nova-Pack was placed between the auto-injector and the column. All the chromatographic information was re-processed in a Star workstation (Version 4.5) supplied by Varian.

Vitamins determination

The contents of liposoluble, water-soluble and B-complex vitamins of the different B. magna samples were quantified according to Albalá-Hurtado et al. (2000). Analyzes were performed in an HPLC system (Hewlett-Packard, Waldbronn, Germany) which consisted of an HP 1050 series de-gasification device, a HP 1100 auto-sampler (for the analysis of water-soluble vitamins), or a Waters 717 (for fat-soluble vitamins analysis) (Waters, Milford, MA, USA). Both equipped with a fixed loop injector of 20 μL, and a UV detector in series HP 1050 of variable wavelength. Water-soluble vitamins were determined from 8 g of sample (in dry basis, for each of the stages of insect development) with 10 mL of Milli-Q water to a 10 mL volumetric flask. The mobile phase used in the HPLC described above contained 5 mM octanesulfonic acid (ion pairing reagent), 0.5% trimethylamine, 2.4% glacial acetic acid, and 15% of methanol in double-distilled water. Nicotinamide, pyridoxine dihydroclorhidre, riboflavin, folic acid, and thiamin hydrochloridre were from Sigma Chemical Co. (St. Louis, MO, USA). Both equipped with a fixed loop injector of 20 μL and ascorbic acid as an antioxidant. The extracts were injected into the HPLC system described above. Water-acetonitrile-methanol (4:1:95, v/v/v) was used as the mobile phase. Working conditions involved dim light and nitrogen atmosphere in order to avoid vitamin degradation. All-trans-Retinol (vit. A) and dl-Tocopherol (vit. E) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The relative standard deviation was lower than 3.5% for both vitamins, and the recovery was higher than 85%.

The data acquisition was performed with a system Chemstation HP 3365-II (Hewlett-Packard). The separation was carried out using a C18 column in reverse phase Tracer Spherisorb ODS2 C18 (TR-011019) de 250 × 4.6 mm, with a particle diameter of 5 μm (Teknokroma, Barcelona, España), with a protective cartridge. Analyzes were performed isocratically with a flow speed of 1 mL/min.

Statistical analysis

For each determination, a minimum size of three replicates (n ≥ 3) was used in all samples. For the analysis of results, a one-way analysis of variance (ANOVA, P ≤ 0.05) was applied using the statistical program MiniTab, version 17 (Minitab Inc., State College, Pennsylvania, USA). The differences between treatments/samples were determined by Tukey’s test (Walpole et al., 1999).

RESULTS AND DISCUSSION

Quantification and molecular weight of proteins

The results of protein content in Brachystola magna at different stages of insect development indicated significant increases (P < 0.05) from ≈ 24% (egg) to ≈ 59% (adult) without significant differences (P > 0.05) between the stages of nymph 3 and nymph 4 (Table 1). These values
are slightly lower than those recently reported by Monter-
Miranda et al. (2018) in similar samples of B. magna, with
values ranging from ≈ 62% (for the stages of development
of nymph 3 and nymph 4) to ≈ 65% for the adult stage
of insect development; however, these investigators did
not determine the content of protein in the egg stage in
the insect B. magna nor determined the molecular weight
of proteins quantified at different stages of development
B. magna. According to these results, it was observed that
in the egg stage of B. magna there is a relatively important
amount of proteins, which can be linked to the exoskeleton
that covers the ootheca (egg sac) and could even be linked
to other structural components such as chitin (Barker et al.,
1998; Mena-Covarrubias, 2009; Yi et al., 2013). The analysis
of SDS-PAGE allowed classifying the proteins according to
their molecular weight in four groups comprised between
10-25 kDa, 25-50 kDa, 100-125 kDa and 150-200 kDa
(Table 1) according to the appearance of various bands
(images not shown). Variations were observed between
the molecular weights of the different bands as a function
of insect development.

The data in relation to the molecular weight of B. magna
proteins are null or scarce. Mishyna et al. (2018) reported
the molecular weights of the soluble proteins of two
edible insects belonging to two different orders, one was
the orthoptera Schistocerca gregaria (a grasshopper, as in this
study) and the other was the hymenopter known as the
honey bee (Apis mellifera). These researchers subjected the
native proteins of these insects to degreasing treatments,
alkaline extractions and sonication, and observed the
presence of bands, which were classified into eight groups
of different molecular weight (10-25, 25-50, 50-75, 75-100, 100-125, 125-150, 150-200 and 200-250 kDa). The
bands that predominated in both insects were those of
10-15 kDa, 25-50 kDa, 50-75 kDa; while the band at 200-
250 kDa was only observed in S. gregaria in its native state.
In accordance with Mishyna et al. (2018) proteins of low
molecular weight in S. gregaria could correspond to fatty
acids linked with proteins involved in the muscular flight
of this orthoptera. This would explain the predominance
of the low molecular weight bands in the nymph and
adult stages of the development of B. magna; however,
future studies are needed to explain the bands with higher
molecular weights.

Amino acid profile
The amino acid profile at the various stages of the
development of B. magna is shown in Fig. 1 At all stages
a relatively high amount of glutamic acid (a non-essential
amino acid) was observed with values ranging from 7.60%
(egg) up to 11.90% (nymph 3), with intermediate values
in stage nymph 2 (≈ 9.00%) and adult (≈ 10.00%). These
values are slightly higher than those recently reported in a
study in which the nutritional composition of five edible
insects were evaluated [(three species of larvae of beetles,
Allemyrina dichotoma (8.69%), Protaetia brevitarsis (5.54%), and
Tenebrio molitor (5.78%), and two species of adult crickets,
Teleogryllus emma (6.51%) and Gryllus bimaculatus (6.39%)] in
South Korea (Ghosh et al., 2017).

In all stages of the development of B. magna, the presence
of 9 out of the 10 essential amino acids (isoleucine,
leucine, lysine, methionine, phenylalalnine, threonine, valine,
histidine, arginine and tryptophan) was detected, and only
the amino acid of tryptophan was not detected. Significant
increases (P < 0.05) were observed in all the essential amino
acids as the growth stage of B. magna increased, which was
related to the greater development of exoskeletal structures
and with the highest content of structural proteins
quantified in the analysis proximal chemical (Table 1).
Some studies have reported that amino acid contents vary

---

**Fig 1.** Brachystola Magna amino acids profile(%) during its development
considerably among different insect species (Bosch et al., 2014; Zielińska et al., 2015; de Castro et al., 2018). In this sense, some authors attribute these differences (besides the type of habitat and feeding of the insect) to the genetic variation that may even exist between species of the same family (Ramos-Elorduy et al., 2012; Makkar et al., 2014; Monter-Miranda et al., 2018).

**Vitamins quantification**

For more than a decade it has been reported in the scientific literature that insects have a wide variety of water-soluble and lipid-soluble vitamins (Finke, 2002, 2005; Xiaoming et al., 2010; Oonincx and Dierenfeld, 2012; Kouřimská and Adámková, 2016). The contents of them are shown in Table 2. Among the water-soluble vitamins, the relatively greater amount of vitamin C in relation to the content of the other water-soluble vitamins is highlighted (Table 2). Vitamin C content increased with the development of *B. magna* with values ranging from ≈ 19.9 mg/100 g (egg) to ≈ 34.6 mg/100 g (adult). These contents are similar to those reported (from 23.8 to 25.5 mg/100 g) in 25 edible species of the order orthoptera (studied in the stage of larvae and adults) collected from different parts of Mexico (Ramos-Elorduy et al., 2012). Regarding to the content of the remaining water-soluble vitamins (vitamins of the B complex), relatively low amounts were observed, and these quantities remained constant during the stage of insect development (Table 2). The values reported in the Table 2 for *B. magna* during all its stages of development were lower than those recorded by Kinyuru et al. (2010) for niacin (3.01-3.22 mg/100 g), riboflavin (0.84-0.96 mg/100 mg) and folic acid (0.34-0.35 mg/100 g) in green and brown grasshoppers (*Raspalia differentia*). On the other hand, the content of pyridoxine during all stages of development of *B. magna* was higher than that reported for green (0.40 mg/100 g) and brown (0.14 mg/100 mg) grasshoppers (*R. differentia*). Recently it has been reported that insects can not synthesize the 8 B-complex vitamins that function as co-enzymes in several required enzymatic reactions, so it has been hypothesized that most insects get their vitamin B requirements from the diet, of microbial symbiosis, or some combination of these complementary sources (Douglas, 2017). If one starts from the above hypotheses (namely, that insects can not synthesize the B-complex vitamins), then, it would have to be assumed that the quantity of B vitamins quantified in the egg stage of the insect would be a product of the structure of the sack of the ootheca (bag in which the eggs are found) and that, later, when *B. magna* hatches and develops in the nymphal and adult stage, it would obtain the requirements of vitamin B from its environment (as previously was mentioned), in any way, the ability to obtain B vitamins and their use in their cellular metabolism would make the levels of this B vitamins complex remain constant. As for fat-soluble vitamins, the content of vitamin A, D and E increased significantly (*p*<0.05) with the stage of development of *B. magna*. This increase in lipid-soluble vitamins was directly proportional to the lipid content previously reported in *B. magna* (Monter-Miranda et al., 2018), where a higher lipid content was observed with the development of the insect. In this regard, Melo-Ruiz et al. (2013) reported that fat-soluble vitamins are part of the lipid content of the insect. A high amount of vitamin E increased across the developmental stages of *B. magna* (21.930-145.360 mg/100 g). This vitamin E content was higher than reported for escamoles ant eggs (*Liometopum apiculatum*) (2.22 mg) (Melo-Ruiz et al., 2013) and crickets (*Acheta domesticus*) (33.13 mg/100 g) (Ayieko et al., 2016). The content of vitamin E in egg and nymph 3 of *B. magna* was in the same order that reported by Kinyuru et al. (2010) for green (16.145 mg/100 g) and brown (17.060 mg/100 g) grasshopper (*Raspalia differentia*), but the it was much higher in nymph 4 and adult (Table 1). Similar to the vitamin E, the vitamin D content increased across the different life cycles of the grasshoppers ranged from 2.710 to 5.250 mg/100 g. These values were greater than reported for escamoles ant eggs (*L. apiculatum*) (0.00361 mg) (Melo-Ruiz et al., 2013). And finally, with respect to vitamin A, the values ranged from 0.150 to 0.390 mg/100 g, in the egg sample.

### Table 2: Vitamin content (mg/100 g) of *Brachystola magna* during its development

| Analysis | Vitamin content* |
| --- | --- |
| **Water-soluble vitamins** | Egg | Nymph 3 | Nymph 4 | Adult |
| Niacin (B<sub>3</sub>) | 0.540±0.060<sup>a</sup> | 0.620±0.100<sup>a</sup> | 0.730±0.130<sup>a</sup> | 0.670±0.220<sup>a</sup> |
| Thiamin (B<sub>1</sub>) | 0.075±0.020<sup>a</sup> | 0.080±0.070<sup>a</sup> | 0.065±0.050<sup>a</sup> | 0.090±0.070<sup>a</sup> |
| Pyridoxine (B<sub>6</sub>) | 0.430±0.030<sup>a</sup> | 0.550±0.030<sup>a</sup> | 0.630±0.040<sup>a</sup> | 0.580±0.090<sup>a</sup> |
| Folic acid (B<sub>9</sub>) | 0.020±0.008<sup>a</sup> | 0.025±0.008<sup>a</sup> | 0.019±0.009<sup>a</sup> | 0.023±0.010<sup>a</sup> |
| Riboflavin (B<sub>2</sub>) | 0.018±0.005<sup>a</sup> | 0.021±0.010<sup>a</sup> | 0.024±0.070<sup>a</sup> | 0.027±0.080<sup>a</sup> |
| Vitamin C | 19.860±1.000<sup>d</sup> | 21.560±0.590<sup>d</sup> | 27.780±0.610<sup>d</sup> | 34.550±0.910<sup>d</sup> |
| **Fat-soluble vitamins** | | | | |
| Vitamin A | 0.150±0.030<sup>c</sup> | 0.310±0.040<sup>c</sup> | 0.310±0.030<sup>c</sup> | 0.390±0.050<sup>c</sup> |
| Vitamin D | 2.710±0.080<sup>d</sup> | 2.340±0.020<sup>d</sup> | 4.370±0.020<sup>d</sup> | 5.250±0.060<sup>d</sup> |
| Vitamin E | 21.930±0.310<sup>d</sup> | 37.110±0.240<sup>d</sup> | 130.710±0.630<sup>d</sup> | 145.360±0.710<sup>d</sup> |

*Arithmetic mean of three determinations±standard error. Equal letters in the same column are not statistically significant (p>0.05).
a significantly smaller quantity \( (P < 0.05) \) was observed as compared with the nymph 3, nymph 4 and adult stages. There were no significant differences between nymph 3 and nymph 4, and the highest value was observed in adult stage. These values were greater than reported in green \( (0.106 \text{ mg/100 g}) \) and brown \( (0.221 \text{ mg/100 g}) \) grasshopper \( \text{(Ruspolia differens)} \) \( (\text{Kinyuru et al., 2010}) \).

The vitamins content and other nutrients in insects is very varied and it is difficult to make comparisons \( (\text{Payne et al., 2016}) \). Further, studies carried out according to the stages of development in this type of insects are scarce, and most of them have been focused on the larval and adult stages of edible insects \( (\text{Hyun et al., 2012; Oonincx and Dierenfeld, 2012; Melo-Ruiz et al., 2013; Kourimská and Adámková, 2016}) \). In addition, the state of development of the insects analyzed is not specified in some studies and the techniques used in their analysis may vary with the authors. On the other hand, the content of nutrients in insects also be strongly influenced by their state of development, depends on each species, seasonality and feeding, which are critical factors in wild insects.

The results of this study of vitamin content during different life cycle of \( \text{B. magna} \) suggest that this grasshopper could be an important source of water-soluble or lipophilic vitamins in diets with reduced consumption of meat products and fruits \( (\text{Millward and Garnett, 2009}) \).

In another hand, as previously mentioned in the introduction, the results generated in this study are complementary to those previously published by our research group \( (\text{Monter-Miranda et al., 2018}) \). In this sense, we have made a diagram of the life cycle of \( \text{B. magna} \) indicating which state of development is the best for each type of nutrient \( (\text{Fig. 2}) \). In general, nymph 3 presents the best source of ash, nymph 4 showed the highest concentration of carbohydrates, while the adult was the best source of proteins and lipids. The eggs and nymph 4 are the main sources of polyunsaturated fatty acids (arachidonic acid). However, \( \text{B. magna} \) eggs represent the best source of monounsaturated fatty acids (oleic and palmitoleic acid). On the other hand, B-complex vitamins were found in the same concentration during all the stages of development evaluated. Regarding the rest of the
In the adult stage of development of *Brachystola magna*, the highest values of protein content (> 59%) were observed in comparison with the other stages of development. In all stages of *B. magna* the presence of 9 out of the 10 essential amino acids was detected (only tryptophan was absent). All the fat-soluble vitamins and vitamin C increased, while the B-complex vitamins remained constant as the stage of development of *B. magna* increased. Due to the presence of proteins, essential amino acids and vitamins it is suggested that this insect could be a potential source of consumption for people with specific health needs. The results of this study and those previously reported by our working group resulted in a complete characterization of the main nutrients present in *B. magna* during different stages of development. Based on this, we can say that this grasshopper could be an excellent source of saturated and unsaturated fatty acids, high-quality protein due to its content of essential amino acids, with a significant contribution of minerals and vitamins. The adult specimens were the best source for most of the nutrients evaluated in the grasshopper.

**CONCLUSIONS**

**ACKNOWLEDGMENTS**

Special recognition to the technical support of Ing. Arturo Ramos Martínez and the work team formed to obtain the insect samples. This study is a product of the Research Group in Carbohydrates, Packaging and Functional Foods (CEAF) of CIAD-Cuauhtemoc, Chihuahua, Mexico, led by Dr. Paul Baruk Zamudio Flores.

**Authors contributions**

PB. Zamudio-Flores (the corresponding author) designed the research plan, execution of experimental work, interpretation of results. J.M. Tirado-Gallegos contributed to the writing of the manuscript and designed the Figures. M. Espino-Díaz, E. Ochoa-Reyes and F. Hernández-Centeno participated in the experimental design. M. Hernández-González and H. Yajaira López-De la Peña, R. Salgado-Delgado, V.G. García-Cano and O. Sánchez-Ortíz performed some analysis and contributed in the translation of the manuscript.

**REFERENCES**

Albalá-Hurtado, S., M. T. Veciana-Nogués, M. C. Vidal-Carou and A. Mariné-Font. 2000. Stability of vitamins A, E, and B complex in infant milks claimed to have equal final composition in liquid and powdered form. J. Food Sci. 65: 1052-1055.

AOAC. 2002. Official Methods of Analysis. 17th ed. AOAC International, Gaithersburg, MD, USA.

Ayieko, M. A., H. Ogola and I. Ayieko. 2016. Introducing rearing crickets (gryllids) at household levels: Adoption, processing and nutritional values. J. Insects Food Feed. 2: 203-211.

Barker, D., M. P. Fitzpatrick and E. S. Dierenfeld. 1998. Nutrient composition of selected whole invertebrates. Zoo Biol. 17: 123-134.

Bosch, G., S. Zhang, D. G. A. Oonincx and W. H. Hendriks. 2014. Protein quality of insects as potential ingredients for dog and cat foods. J. Nutr. Sci. 3: e29.

Bustillos-Rodríguez, J. C., C. Rios-Velasco, R. Valdés-Licano, D. I. Berfanga-Reyes, J. O. Omalas-Paz, C. H. Acosta-Muñiz, M. F. Ruiz-Cisneros, M. A. Salas-Marina and O. J. Cambero-Campos. 2016. Laboratory assessment of *Metarhizium* spp. and *Beauveria* spp. isolates to control *Brachystola magna* in Northern México. Southwest. Entomol. 41: 643-656.

de Castro, R. J. S., A. Ohara, J. G. D. Aguilar and M. A. F. Domingues. 2018. Nutritional, functional and biological properties of insect proteins: Processes for obtaining, consumption and future challenges. Trends Food Sci. Technol. 76: 82-89.

Douglas, A. E. 2017. The B vitamin nutrition of insects: The contributions of diet, microbiome and horizontally acquired genes. Curr. Opin. Insect Sci. 23: 65-69.

Finke, M. D. 2002. Complete nutrient composition of commercially raised invertebrates used as food for insectivores. Zoo Biol. 21: 269-285.

Finke, M. D. 2005. Nutrient content of insects. In: J. L. Capinera (Ed.), Encyclopedia of Entomology. 1st ed. Dordrecht, Springer, The Netherlands, pp. 1563-1575.

Ghosh, S., S. M. Lee, C. Jung and V. B. Meyer-Rochow. 2017. Nutritional composition of five commercial edible insects in South Korea. J. Asia Pac. Entomol. 20: 686-694.

González Paramás, A. M. G., J. A. G. Bárez, C. C. Marcos, R. J. García-Villanova and J. S. Sánchez. 2006. HPLC-fluorimetric method for analysis of amino acids in products of the hive (honey and bee-pollen). Food Chem. 95: 148-156.

Hyun, S. H., K. H. Kwon, H. C. Jeong, O. Kwon, H. Tindwa and Y. S. Han. 2012. Evaluation of nutritional status of an edible grasshopper, *Oxya chinensis formosana*. Entomol. Res. 42: 284-290.

Jonas-Levi, A. and J. J. I. Martinez. 2017. The high level of protein content reported in insects for food and feed is overestimated. J. Food Compost. Anal. 62: 184-188.

Khosravi-Largani, M., P. Pourvali-Talapappeh, A. M. Rousta, M. Karimi-Kivi, E. Noroozi, A. Mahjoob, Y. Asaadi, A. Shahrhammadadi, S. Sadeghi, S. Shakeri, K. Ghiyasvand and M. Tavakoli-Yaraki. 2018. A review on potential roles of vitamins in incidence, progression, and improvement of multiple sclerosis. eNeurologicalSci. 10: 37-44.

Kinyuru, J. N., G. M. Kenji, S. M. Njoroge and M. Ayieko. 2010. Effect of processing methods on the *in vitro* protein digestibility and vitamin content of edible winged termite (*Macrotermes*...
subhylanus) and grasshopper (Ruspolia differens). Food Bioprocess Tech. 3: 778-782.

Kouřímská, L. and A. Adámková. 2016. Nutritional and sensory quality of edible insects. NFS J. 4: 22-26.

Löffler, F. and T. Hartmann. 2017. Effects of landscape and habitat quality on Orthoptera assemblages of pre-alpine calcareous grasslands. Agric. Ecosys. Environ. 248: 71-81.

Lozano, G. and L. M. España. 2009. Enemigos naturales y control biológico de Brachystola magna (Girard) y B. mexicana (Bruner) (Orthoptera: Acrididae) con Beauveria bassiana en Zacatecas, México. Vedalia. 13: 91-96.

Luan, J. B., W. Chen, D. K. Hasegawa, A. M. Simmons, W. M. Wintermantel, K. S. Ling, Z. Fei, S. S. Liu and A. E. Douglas. 2015. Metabolic coevolution in the bacterial symbiosis of whiteflies and related plant sap-feeding insects. Genome Biol. Evol. 7: 2635-2647.

Makkar, H. P. S., G. Tran, V. Heuzé and P. Ankers. 2014. State-of-the-art on use of insects as animal feed. Anim. Feed Sci. Technol. 197: 1-33.

Melo-Ruíz, V., T. Quirino-Barreda, C. Calvo-Carrillo, K. Sánchez-Herrera and H. Sandoval-Trujillo. 2013. Assesment of nutrients of escamoles ant eggs Liometopus apiculatum M. by spectroscopy methods. J. Chem. Chem. Eng. 7: 1181-1187.

Mena-Covarrubias, J. 2009. Control biológico del chapulín Brachystola spp. (Orthoptera: Acrididae) con el uso del protozoario Nosema locustae Canning (Microsporidia: Nosematidae) en Zacatecas, México. Vedalia. 13: 97-102.

Millward, D. J. and T. Garnett. 2009. Plenary lecture 3 food and the planet: Nutritional dilemmas of greenhouse gas emission reductions through reduced intakes of meat and dairy foods: Conference on over and undernutrition: Challenges and approaches. Proc. Nutr. Soc. 69: 103-118.

Mishyna, M., J. J. I. Martínez, J. Chen and O. Benjamin. 2018. Extraction, characterization and functional properties of soluble proteins from edible grasshopper (Schistocerca gregaria) and honey bee (Apis mellifera). Food Res. Int. 116: 697-706.

Monter-Miranda, J. G., J. M. Tirado-Gallegos, P. B. Zamudio-Flores, C. Rios-Velasco, J. D. J. Ornelas-Paz, R. Salgado-Delgado, V. Espinosa-Solís and F. Hernández-Centeno. 2016. Extracción y caracterización de propiedades físicoquímicas, morfológicas y estructurales de quitina y quitosano de Brachystola magna (Girard). Rev. Mex. Ing. Quim. 15.

Monter-Miranda, J. G., P. B. Zamudio-Flores, J. M. Tirado-Gallegos, F. J. Molina-Corrall, E. Ochoa-Reyes, C. Rios-Velasco, M. Hernández-González, F. Hernández-Centeno y H. Y. L. de la Peña. 2018. Nutritional characterization of fatty acids and minerals in Brachystola magna (Girard) during their development. Emir. J. Food Agric. 30: 389-395.

Ooincx, D. G. A. and E. S. Dierenfeld. 2012. An investigation into the chemical composition of alternative invertebrate prey. Zoo Biol. 31: 40-54.

Payne, C. L. R., P. Scarborough, M. Rayner and K. Nonaka. 2016. A systematic review of nutrient composition data available for twelve commercially available edible insects, and comparison with reference values. Trends Food Sci. Technol. 47: 69-77.

Ramos-Elorduy, B. J., M. J. M. Pino and C. V. H. Martínez. 2012. Could grasshoppers be a nutritive meal? Food Nutr. Sci. 3: 12.

SAGARPA. (2012). Reglas de Operación de los Programas de la Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, DOF. P. 31.

Snyder, A. K. and R. V. M. Rio. 2015. “Wigglesworthia morsitans” folate (Vitamin B9) biosynthesis contributes to tsetse host fitness. Appl. Environ. Microbiol. 81: 5375-5386.

Walpole, R. E., R. H. Myers and S. L. Myers. 1999. Probabilidad y estadística para ingenieros. Prentice-Hall Hispanoamericana, S.A., México.

Xiaoming, C., F. Ying, Z. Hong and C. Zhiyong. 2010. Review of the nutritive value of edible insects. In: P. B. Durst, D. V. Johnson, R. N. R Leslie and K. Shono (Eds.), Forest Insects as Food: Humans Bite Back. Vol. 85. FAO, Bangkok, Thailand, pp. 85-92.

Yi, L., C. M. M. Lakemond, L. M. C. Sagis, V. Eisner-Schadler, A. van Huis and M. A. J. S. van Boekel. 2013. Extraction and characterisation of protein fractions from five insect species. Food Chem. 141: 3341-3348.

Zielińska, E., B. Baraniak, M. Karaś, K. Rybczyńska and A. Jakubczyk. 2015. Selected species of edible insects as a source of nutrient composition. Food Res. Int. 77: 460-466.

Zielińska, E., M. Karaś and B. Baraniak. 2018. Comparison of functional properties of edible insects and protein preparations thereof. LWT-Food Sci. Technol. 91: 168-174.