PHENOLIC CHARACTERIZATION AND BIOACTIVITY OF MICROWAVE-ASSISTED EXTRACTS FROM EDIBLE HOUSE CRICKETS (*ACHETA DOMESTICUS)*

by

Maria Catalina Nino Bernal

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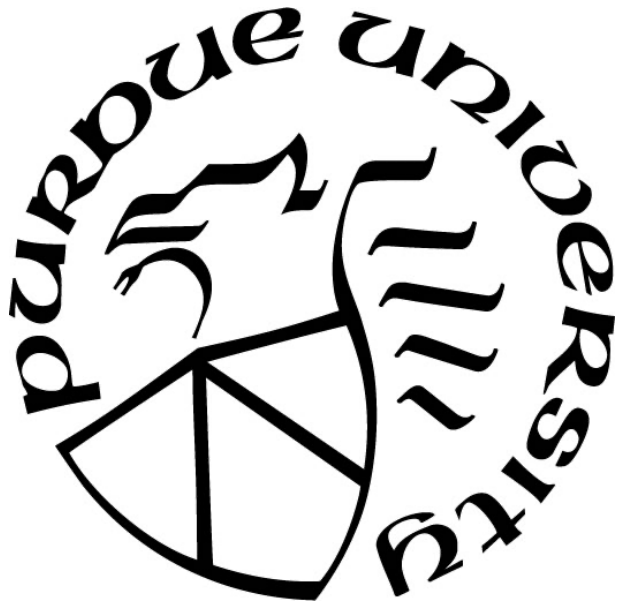

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STATEMENT OF COMMITTEE APPROVAL

Dr. Andrea Liceaga, Chair

Department of Food Science

Dr. Lavanya Reddivari

Department of Food Science

Dr. Coralia Osorio

Department of Chemistry, Universidad Nacional de Colombia

Dr. Ian Kaplan

Department of Entomology

Approved by:

Dr. Arun Bhunia

This thesis is dedicated to my mother Claudia, my father Juan Manuel and my brother Carlos Felipe for their support, inspiration, and immense love.

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ABSTRACT

Entomophagy, which is the habit of eating insects, has become relevant in the past few years as it could potentially help reduce current and future food insecurity, due to the highly nutritious and sustainable characteristics of edible insects. In addition to the nutritional content of insects, research on the potential bioactive components of insect extracts has also gained popularity. In this study, extracts from house cricket (*Acheta domesticus*) from two farms and their corresponding feeds were obtained using a microwave-assisted extraction. Further phenolic characterization led to the identification and quantification of 4-hydroxybenzoic acid, *p*-coumaric acid, ferulic acid and syringic acid as major phenolic compounds in both *A. domesticus* extracts as well as both feed extracts. Additionally, *in vitro* antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl radical cation (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical assays. In both *in vitro* assays, *A. domesticus* extracts showed higher antioxidant activity compared to the feeds. Antibacterial activity against *E. coli* and *L. innocua* was also evaluated using the microwell method. The *A. domesticus* extracts showed a selective inhibition ($p<0.05$) towards the gram-positive bacteria *L. innocua* between a period of 4 to 8 h. This inhibition is thought to have occurred as a result of the presence of phenolic acids and antimicrobial peptides, while the feed extracts did not exhibit any inhibitory activity towards any of the bacteria. The finding of the same phenolic acids in *A. domesticus* and their corresponding feed could imply the capacity of *A. domesticus* to absorb and sequester dietary phenolics that may provide additional health benefits when the insect is consumed, unveiling new benefits of entomophagy.

CHAPTER 1. INTRODUCTION

1.1 Entomophagy and insect nutritional content and bioactivity

The habit of eating insects, entomophagy, has been associated with almost every human community around the world as an ancient cultural practice (Hanboonsong, 2010). It is estimated that more than 1900 different species of insects are consumed in modern day (Hanboonsong, 2010; Van Huis, 2013) in various communities mainly located in Asia, Africa and Latin America, where they are part of the daily diet and are considered a delicacy (DeFoliart, 1999; Van Huis, 2013). Nonetheless, for almost all Western cultures, this practice is still unfamiliar being a cultural taboo limiting its incorporation in the current western diet (Van Huis, 2013). However, efforts to eliminate this taboo are gaining popularity due to the multiple nutritional and promising health benefits that allow entomophagy to be regarded as a sustainable and feasible approach to solve current and future food insecurity as stated by the Food and Agriculture Organization (FAO) (Gahukar, 2011; Payne et al., 2019). Lately, extensive research has been published on the nutritional qualities of popular edible insects (Tropical banded cricket (*Gryllodes sigillatus*), house cricket (*Acheta domesticus*) mealworm (*Tenebrio molitor*) and desert locust (*Schistocerca gregaria)*), indicating that these have high protein content (at least 50 %, dry basis) (da Silva Lucas et al., 2020; Hall et al., 2017; Zielińska et al., 2015). In addition, these insects could be a source of minerals and vitamins (Rumpold & Schlüter, 2013). Furthermore, product development efforts to incorporate insects in foods have shown promising results. Recently, Calzada-Luna et al. (2020) incorporated cricket protein hydrolysates from farmed *A. domesticus* in the formulation of corn tortilla and tortilla chips resulting in final products with sensory acceptability.

Bioactivity of insect extracts and insect protein hydrolysates has also gained significance in recent years. Hall and Liceaga (2020) reported DPP-IV and ACE inhibition activity (IC_{50} = 0.096 mg/ml and 0.27 mg/ml, respectively) of peptides from *G. sigillatus.* In another study, del Hierro et al. (2020) reported DPPH antioxidant activity of *A. domesticus* and *T. molitor* extracts using ultrasound-assisted extraction (UAE), having close to 80 % inhibition of the DPPH radical. Furthermore, antibacterial bioactivity of insect extracts has also been reported. Whole insect extracts of house fly (*Musca domestica*) exhibited relevant antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*, both gram-positive.

Although evidence of bioactivity of insect components and whole insect extracts has been published, the main focus of these studies has been on the protein fractions and peptides. Other bioactive compounds, such as polyphenols have not been extensively addressed, especially for edible insects. *A. domesticus* is one of the most commonly reared cricket species for human consumption in western countries; therefore, interest on the study of bioactive compounds, like phenolics, that could be absorbed from their diet, becomes relevant. The discovery of these dietary phenolics could lead to additional health benefits of entomophagy.

1.2 Objective

The main goal of this research is to demonstrate that edible farmed *A. domesticus* can absorb or sequester phenolic compounds from their diet and that these extracts have bioactive potential. In order to approach this objective, microwave-assisted extraction was used to obtain extracts from farmed *A. domesticus* consuming two different diets after which the phenolic characterization and bioactive potential (*in-vitro* antioxidant and antibacterial bioactivity) of the extracts was evaluated.

1.3 Hypothesis

My hypothesis is that *A. domesticus* extracts will contain dietary phenolic compounds and will exert antioxidant and antibacterial bioactivities *in vitro*.

1.4 References

Calzada-Luna, G., San Martin-Gonzalez, F., Mauer, L., & Liceaga, A. M. (2020). Cricket (Acheta domesticus) Protein Hydrolysates Impact on the Physicochemical, Structural and Sensory properties of Tortillas and Tortilla chips. *Journal of Insects as Food and Feed, In press*.

da Silva Lucas, A. J., de Oliveira, L. M., da Rocha, M., & Prentice, C. (2020). Edible insects: An alternative of nutritional, functional and bioactive compounds. *Food chemistry, 311*, 126022.

DeFoliart, G. R. (1999). Insects as food: why the western attitude is important. *Annual review of entomology, 44*(1), 21-50.

del Hierro, J. N., Gutiérrez-Docio, A., Otero, P., Reglero, G., & Martin, D. (2020). Characterization, antioxidant activity, and inhibitory effect on pancreatic lipase of extracts from the edible insects *Acheta domesticus* and *Tenebrio molitor*. *Food chemistry*, 125742.

Gahukar, R. (2011). Entomophagy and human food security. *International Journal of Tropical Insect Science, 31*(3), 129-144.

Hall, F., & Liceaga, A. (2020). Effect of microwave-assisted enzymatic hydrolysis of cricket (Gryllodes sigillatus) protein on ACE and DPP-IV inhibition and tropomyosin-IgG binding. *Journal of functional foods, 64*, 103634.

Hall, F. G., Jones, O. G., O'Haire, M. E., & Liceaga, A. M. (2017). Functional properties of tropical banded cricket (Gryllodes sigillatus) protein hydrolysates. *Food chemistry, 224*, 414-422.

Hanboonsong, Y. (2010). Edible insects and associated food habits in Thailand. *Forest insects as food: humans bite back, 173*, 182.

Payne, C., Megido, R. C., Dobermann, D., Frédéric, F., Shockley, M., & Sogari, G. (2019). Insects as Food in the Global North–The Evolution of the Entomophagy Movement. In *Edible Insects in the Food Sector* (pp. 11-26). Springer.

Rumpold, B. A., & Schlüter, O. K. (2013). Potential and challenges of insects as an innovative source for food and feed production. *Innovative Food Science & Emerging Technologies, 17*, 1-11.

Van Huis, A. (2013). Potential of insects as food and feed in assuring food security. *Annual review of entomology, 58*, 563-583.

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.

CHAPTER 2. INSECTS AS A SOURCE OF PHENOLIC COMPOUNDS AND POTENTIAL HEALTH BENEFITS

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2.1 Abstract

The use of insects in traditional medicine and unveiling the chemical structure of the bright pigments in butterfly wings led to the discovery of bioactive phenolic compounds in the insect bodies. These metabolites have been found not only due to the insect absorption and metabolization of the plant-derived phenolic present in their diet, but also from the ability of insects to synthesize phenolic compounds *de novo* through the sclerotisation process. Plant phenolics are secondary metabolites involved in the protection of tissues against UV radiation, herbivores, and pathogens, as well as pigmentation of fruits and flowers. These bioactive compounds exhibit antioxidant, anti-inflammatory, anticancer, and antimicrobial activities, demonstrated through *in vitro* and *in vivo* studies. This bioactive potential is thought to occur due to their chemical characteristics that allow them to stabilize reactive oxygen species (ROS), chelate prooxidant metal ions, interact with key enzymes and signal cascades involved in biological pathways. Bioactivity of plant phenolics and both *in vitro*, *in vivo* studies, suggest that the dietary compounds absorbed by the insect maintain their chemical and bioactive properties. Further characterization of the phenolic composition in edible insects and evaluation of their bioactive capacity as well as their bioavailability, could result in discovering additional health benefits of entomophagy apart from macro-nutritional (e.g. protein) content.

2.2 Introduction

Plant phenolic compounds are secondary metabolites characterized by the presence of one or more aromatic rings in their structure with at least one hydroxyl group attached. They can be classified as flavonoids or non-flavonoids according to the number of benzene rings and the type of functional group attached to the aromatic ring (Ignat et al., 2011; Quideau et al., 2011). Their

production in plant tissues has been attributed to diverse functions including resistance against microbial pathogens and viruses, protection from herbivores, protection against solar irradiation, specifically UV rays, and exerting a role in pollination processes (Cheynier, 2012; Quideau et al., 2011). Furthermore, phenolic compounds have been long recognized to have several bioactivities including antioxidant, anti-inflammatory, antimicrobial and anticancer (Carocho & CFR Ferreira, 2013; Di Carlo et al., 1999; Farhadi et al., 2019; Gomes et al., 2008), among others, thus providing health benefits when they are incorporated in human diets.

Given the biochemical and functional diversity of plant phenolics, herbivorous insects that consume these compounds from leaves and other plant tissues should retain some of their bioactive properties. During the mid-20th century, scientists discovered the presence of phenolic compounds in the insect cuticle, wings, and intestinal tract, hypothesizing that these were absorbed and/or metabolized from the diet and later incorporated in the body (Simmonds, 2003).However, phenolics in edible insects remain to be characterized or if insects are potential sources of polyphenols, which would further increase the health benefits of entomophagy. The aim of this review is to integrate the scientific findings regarding the presence of phenolic compounds in insects and their potential health benefits.

2.3 Insect phenolics

2.3.1 Sclerotisation as a source of phenolic compounds

Sclerotization is the process by which the insect cuticle is hardened as a result of the incorporation of phenolic compounds in the cuticular matrix involving structural proteins and chitin, through a series of enzyme-mediated reactions (Andersen, 2010). Although the occurrence of phenolics in insects is strongly related to their diet, research on the chemical mechanisms of sclerotisation has proven that non-dietary phenolic compounds are also present in the insect body. These non-dietary phenolics are synthesized through enzyme-mediated reactions taking place in the cuticle where phenoloxidase enzymes play a major role (Sugumaran, 2010).The incorporation of these compounds in the insect cuticle results in the stabilization and hardening of the cuticular structure (Andersen, 2010; Mun et al., 2015). The insect cuticle acts as a mechanical support of various body parts and protects the insect from foreign substances, e.g. pathogens (Sugumaran, 1998). The insect cuticle consists of two main parts, the procuticle composed of chitin filaments

organized in a protein network, and the epicuticle consisting of lipid and protein arrangements. The hard characteristic of the cuticular structure prevents the insect from growing and thus at every stage of its life cycle, the insect sheds the old cuticle and synthesizes a new one that will undergo sclerotisation. The general suggested mechanism (Figure 1) starts with tyrosine to synthesize the two acyldopamine precursors, N-acetyl dopamine (NADA) and N-*β*-alanyl dopamine (NBAD) that will undergo oxidation generating the corresponding quinones (Moussian, 2010). Consequently, NADA-*para*-quinone methide can be enzymatically rearranged to a side chain unsaturated catechol, *α,β*-dehydro-NADA (Andersen & Roepstorff, 1982) that can be further oxidized, producing unsaturated quinoid derivatives that react with available catechols and then react with amino acid residues of different proteins in the cuticle thereby generating the crosslink (Andersen & Roepstorff, 2007). Although the primary crosslink may occur between the unsaturated quinoid derivatives and two amino acid residues, adducts between N-acyldopamines and amino acid residues can be formed at previous stages. Following the oxidation of the N-acyldopamines, the *ortho*-quinones and the isomer *para*-quinone methides, may react with nucleophilic amino acid residues resulting in acyldopamines substituted in the 6-position and in the *β*-position of the side chain, respectively (Andersen, 2010). Although this represents the current understanding of the major chemical reactions involved in sclerotisation, further research is needed to confirm the exact pathway involved. In addition, it is possible that different catechols, other than NADA and NBAD, contribute to the sclerotisation process as the specific path varies among insects (Andersen, 2010). Furthermore, the bioavailability of these phenolic compounds has to be determined as well as their contribution to the total phenolic content and their possible human health benefits.

2.3.2 Impact of diet on insect phenolic composition

From the diversity of plant phenolics that insects encounter, flavonoids appear to be the most commonly absorbed (Simmonds, 2003). Flavonoids constitute a variety of low molecular weight compounds arranged in a C6-C3-C6 structure, consisting of two phenolic benzene rings A and B joined by a three-carbon bridge usually forming a heterocyclic ring C (Ignat et al., 2011; Shahidi & Yeo, 2018). The aromatic ring A originates from the acetate/ malonate pathway while ring B is derived from the shikimate pathway. Variations in the structure of ring C lead to further

classification of flavonoids into flavones, flavonols, flavanones, flavanols, isoflavones, and anthocyanins (Ignat et al., 2011)

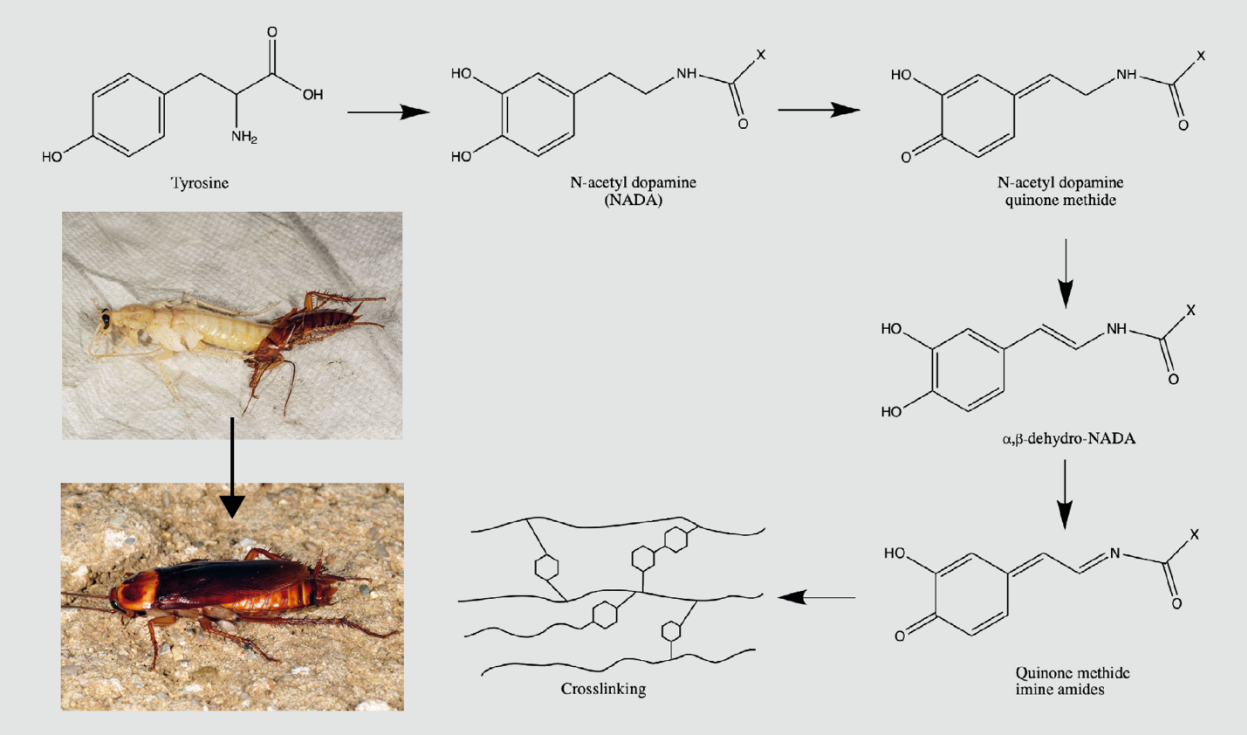

Figure 1. Simplified suggested mechanism of the sclerotisation of insects (photo credit: John Obermeyer, Purdue University).

The great majority of phenolic compounds found in insects are attributed to herbivore feeding behavior. Research in the metabolism of phenolic compounds derived from the insect diet has been of interest since the early 20th century (Burghardt et al., 1997; Ferreres et al., 2009a; Harborne, 1991; Musundire et al., 2014b; Salminen & Lempa, 2002; Schittko et al., 1999).

For example, interest in the characterization of butterfly wing pigments led Thomson (1926) and subsequent researchers to show the presence of phenolic compounds as part of the wing structure. Thomson (1926) was a pioneer in revealing the presence of flavonoids as part of the structure of the wings of a marbled white butterfly (*Melanargia galathea*), which were thought to act as UV-light protectors and were observed in other diurnal species. Years later, Morris and Thomson (1963) identified the *O*-methylated flavone tricin (Figure 2) as the main phenolic compound in *M. galathea* as well as tricin glycosides, which were thought to be absorbed from the insect diet. Further studies on the phenolic composition of *M. galathea* and other lepidopterans

(family Lycaenidae) have supported the hypothesis of the presence of flavonoids as a result of the sequestration from their host plants, as well as the insect capacity to metabolize these compounds yielding flavonoids that are not found in the plant (Wilson, 1985, 1987). Wiesen et al. (1994) reported the phenolic profile of the common blue butterfly (*Polyommatus icarus*), characterized by twelve different flavonoids of *P. icarus* reared on inflorescences of purple crown vetch (*Coronilla varia*) and alfalfa (*Medicago sativa*) by using spectroscopic techniques (NMR, MS). Larvae, pupae, and adult *P. icarus* reared on crown vetch and alfalfa showed a similar phenolic profile to that of the specific host plant having the flavonoid kaempferol-3-*O*-glucoside as a primary compound. Also, kaempferol-3,7-di-*O*-glucoside was detected in the larvae, which is believed to be a biotransformation product from kaempferol (Figure 2) or kaempferol-3-*O*-glucoside in plants. These results showed selectivity for the absorption of kaempferol and kaempferol glucosides over quercetin and myricetin that are also present in both host plants. Overall, *P. icarus* larvae have an average flavonoid concentration of 36 mg/g dry weight DW, whereas pupae and adults had an average of 35-45 mg/g DW. In *P. icarus* adults, 80 % of the flavonoids were located in the wings while the remaining 20 % was distributed in the body. Furthermore, larvae were fed with an artificial diet containing kaempferol as the only source of phenolic compounds. Subsequent analysis of these larvae showed the presence of kaempferol-3-*O*-glucoside, indicating the ability of insects to glycosylate flavonols. It appears that the formation of glucosides is a common metabolic pathway of flavonoid metabolism in insects (Hirayama et al., 2008; Lahtinen et al., 2006; Salminen et al., 2004). Interestingly, the qualitative and quantitative phenolic compound profiles of males and females were not different. In contrast, Burghardt et al. (1997) observed a higher quantity of flavonoids in adult females, having 37.2 % more than males of *P. icarus* reared on fodder vetch (*Vicia villosa*). Phenolic composition of fully grown fourth-instar caterpillars and adult butterflies consisted of the three main flavonoids (myricetin-3-O-rhamnoside, quercetin-3-O-rhamnoside, kaempferol-3- O-rhamnoside) that were found in fodder vetch. Selective uptake of kaemferol-3-*O*-rhamnoside and only trace amounts of myricetin-3-*O*-rhamnoside in adult butterflies were found. Superior flavonoid sequestration by the female butterflies of this species and their allocation in the wings appears to influence the behavior of mate-searching males, indicating a possible preference for females with higher flavonoid content (Burghardt et al., 2000). Later, Burghardt et al. (2001) assessed the flavonoid content of individuals of *P. icarus* reared on ten different host plants, five of which are known to be natural

feeding plants of wild *P. icarus,* and the other half corresponded to plant species or plant organs that are not natural hostplants of *P. icarus*. In this study, a direct relationship between the flavonoid content of the insect and feed source was observed, thus evidencing the impact of the diet. Moreover, larvae showed the capacity to absorb dietary phenolics from the non-habitual hostplants, although at a reduced quantity than the larvae fed with the natural hostplants. Also, selective absorption of quercetin and kaempferol was observed as well as a higher flavonoid absorption capacity of female individuals.

In another study, Schittko et al. (1999) confirmed the sequestration and absorption of phenolic compounds by *P. icarus* reared on inflorescences of white clover (*Trifolium repens*). The *P. icarus* flavonoid characterization showed quercetin-3-*O*-galactoside as the main component, as well as the main phenolic compound in the host plant. In addition, the average content of flavonoids was higher for females (5.85 μg/ mg DW) than for males (3.49 μg/mg DW), showing the same sexual variation reported by Burghardt et al. (1997). This fact supports the hypothesis of flavonoids play a role in wing pigmentation. Flavonoid content also correlated with the dry weight of the insect, increasing with insect developmental stages, in accordance with an enrichment process. Geuder et al. (1997) reported a selective uptake of flavonoids (flavone C-glycosides) from the leaves of the purple crown vetch (*C. varia*), by larvae, pupae, and adults of the Adonis blue butterfly (*Polyommatus bellargus*), followed by a bioconversion into the corresponding flavonol glycosides. In this case, a comparative HPLC analysis demonstrated that *P. bellargus* absorbed isovitexin from the plant, with isovitexin-2'-*O*-xyloside identified as the major constituent. *P. bellargus* phenolic compounds were predominantly located in the wings, supporting the hypothesis that phenolics act as UV-shields in diurnal butterflies.

In addition to the research with lepidopterans, flavonoid pigments have also been studied for Carolina locust (*Dissoteira carolina*). The characterization of the yellow pigments in the wings of *D. carolina* resulted in the identification of quercetin and quercetin-3-*β*-*O*-glucoside was attributed to the absorption of quercetin glycosides from the plant tissues (Hopkins & Ahmad, 1991). Most recently, Hirayama et al. (2013) isolated two flavonol glycosides: quercetin 3-*O*-*β*-*D*-galactopyranosyl-(1→3)-*β*-*D*-galactopyranoside and kaempferol 3-*O*-*β*-*D*-galactopyranosyl-(1→3)-*β*-*D*-galactopyranoside, as well as four other flavonoids from the cocoon of the white caterpillar (*Rondotia menciana*) that were fed exclusively with mulberry (*Morus alba*) leaves. These two flavonol glycosides were not found on the mulberry leaves, indicating the

metabolization of dietary flavonoids by the insect for further incorporation in the cocoon. Similar studies have shown the presence of flavonoids in the cocoon of silkworm (*Bombyx mori)*, confirming the absorption of these bioactive compounds from the plant and their subsequent modification (Hirayama et al., 2006; Kurioka & Yamazaki, 2002; Tamura et al., 2002). Ferreres et al. (2009b) analyzed the phenolics from tronchuda cabbage (*Brassica oleracea* var. *costata*) when ingested by larvae of the large white butterfly (*Pieris brassicae*), which were metabolized, undergoing diacylation and sulfation. Previous studies on *P. brassicae* showed the insect biotransformation capacity of other host plant phenolics and the influence that dietary phenolic composition has on the metabolic pathway developed by *P. brassicae* (Ferreres et al., 2009a; Ferreres et al., 2008).

To this point, little is known of the phenolic composition on edible insects. Recently, raw and traditionally processed (cooked in warm water followed by heat drying) edible stink bugs (*Encosternum delegorguei*) were analyzed to quantitate bioactive compounds of this popular insect consumed in Zimbabwe (Musundire et al., 2014a). Unprocessed insects showed higher amounts of total phenolics (3.6 g of gallic acid equivalents (GAE)/100 g), tannins, and flavonoids (0.31 g of catechin equivalents (CE)/100 g and 15.20 g CE/100 g, respectively) than processed insects (total phenolics 2.8 g GAE/100 g, tannins 0.10 g CE/100 g, and flavonoids 4.80 g CE/100 g). This is likely due to degradation of phenolic compounds during the heating process, as well as the loss of the compounds in aqueous media while cooking. A similar study with the edible beetle *Eulepida mashona* showed that different preparation methods also affected the nutritional and bioactive compounds' content of the insect (Musundire et al., 2016). Total phenolics and flavonoids content decreased when the insect was cooked for 10 min and 20 min, respectively. However, a much more significant change in total phenolics was observed for 30 min cooking (total phenolics 0.20 mg GAE/1 g DW) compared to the dried uncooked sample (total phenolics 0.81 mg GAE/1 g DW). In another study, Musundire et al. (2014b) identified the presence of phenolic compounds in edible ground cricket (*Henicus whellani*) from Zimbabwe, and quantified the total phenolics (7.7 mgGAE/g), flavonoids (15.5 mg CE/g), and tannins (0.17 mg CE/g), indicating that *H. whellani* was able to absorb these compounds from plant sources and sequester or metabolize them.

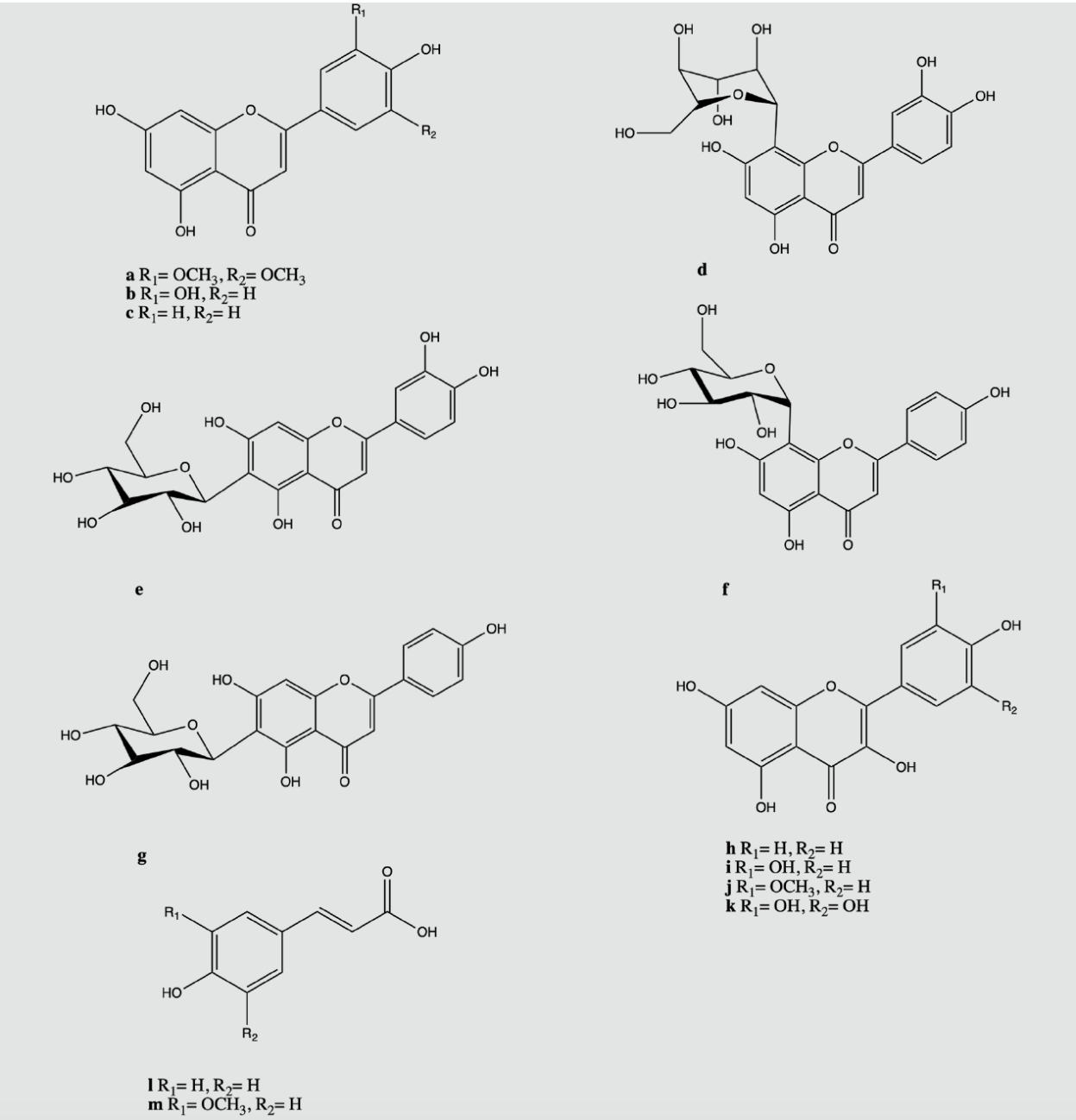

Figure 2. Phenolic compounds detected in insects: (a) Tricin, (b) luteolin, (c) apigenin, (d) orientin, (e) iso-orientin, (f) vitexin, (g) isovitexin, (h) kaempferol, (i) quercetin, (j) isorhamnetin, (k) myricetin, (l) ferulic acid, (m) sinapic acid.

Table 1. Phenolic compounds reported for different insect species.

Insect species	Identified phenolic compounds	Location of phenolics in the insect	Reference
Marbled white butterfly (*Melanargia galathea*)	Flavones: tricin, tricin 7-glucoside, tricin 7-diglucoside, tricin 4'-glucoside, luteolin, luteolin 7-glucoside, luteolin 7-diglucoside, luteolin 7-triglucoside, apigenin, apigenin 7-glucoside, orientin, orientin 7-glucoside, iso-orientin, iso-orientin 7-glucoside, vitexin 7-glucoside, vitexin 7-glucoside, isovitexin, isovitexin 7-glucoside	wings and body	Morris and Thomson, 1963; Thomson, 1926; Wilson, 1985
Halkhill blue butterfly (*Lysandra coridon* Poda)	Flavonols: kaempferol, kaempferol 7-rhamnoside, kaempferol 3-rhamnoside, kaempferol 3-glucoside, kaempferol 3-glucoside, 7-rhamnoside, quercetin 3-glucoside, quercetin 3,7-diglucoside, isorhamnetin 3-glucoside, isorhamnetin 3,7-diglucoside	wings and body	Wilson, 1987
Common blue butterfly (*Polyommatus icarus*)	Flavonols: quercetin, kaempferol, kaempferol 3-*O*-glucoside, kaempferol 3-*O*-(6'-malonyl) glucoside, kaempferol 3-*O*-galactoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, kaempferol 3,7-*O*-diglucoside, myricetin-3-*O*-rhamnoside, quercetin-3-*O*-rhamnoside, kaempferol-3-*O*-rhamnoside	wings and body	Burghardt *et al.*, 1997, 2001; Schittko *et al.*, 1999; Wiesen *et al.*, 1994; Wilson, 1987
Adonis blue butterfly (*Polyommatus bellargus*)	Flavones: isovitexin-2'-*O*-xyloside, iso-orientin Flavonols: kaempferol and quercetin glycosides	wings and body	Geuder *et al.*, 1997
Carolina locust (*Dissoteira carolina*)	Flavonols: Quercetin, quercetin-3-*β*-*O*-glucoside	wings	Hopkins and Ahmad, 1991
Mulberry white caterpillar (*Rondotia menciana*)	Flavonols: quercetin 3-*O*-galactosyl-galactoside, quercetin-3-*O*-galactoside, kaempferol 3-*O*-galactosyl-galactoside, kaempferol 3-*O*-galactoside, quercetin 3-*O*-β-*D*-galactopyranosyl-(1→3)-*β*-*D*-galactopyranoside, kaempferol 3-*O*-*β*-*D*-galactopyranosyl-(1→3)-*β*-*D*-galactopyranoside	cocoon	Hirayama *et al.*, 2013
Silkworm (*Bombyx mori*)	Flavonols: Quercetin, kaempferol, quercetin 5-*O*-*β*-D-glucoside, quercetin 7-*O*-*β*-D-glucoside, quercetin 4'-*O*-*β*-D-glucoside, kaempferol 5-*O*-*β*-D-glucoside, kaempferol 7-*O*-*β*-D-glucoside, quercetin 5-glucoside, quercetin 5,4'-diglucoside, quercetin 5,7,4'-triglucoside	cocoon	Kurioka and Yamazaki, 2002; Tamura *et al.*, 2002
Large white butterfly (*Pieris brassicae*)	Flavonols: kaempferol-3-*O*-sophoroside-7-*O*-glucoside, kaempferol-3-*O*-sophoroside Phenolic acids: ferulic and sinapic acids	larvae and adult body	Ferreres *et al.*, 2008, 2009b
Dark black chafer beetle (*Holotrichia parallela*)	Flavanol: catechin	adult body	Liu *et al.*, 2012

[1] Structure of phenolic compounds are shown in Figure 2.

The aforementioned research demonstrates the presence of phenolic compounds in insects (primarily lepidopterans) as a result of the absorption of dietary phenolics, as well as their capacity to metabolize these compounds and incorporate them into their structure. Remarkably, insects appear to have a selective uptake of flavonols, mainly kaempferol and quercetin, as well as flavones such as tricin and isovitexin (Table 1). Most of them are glycosylated with only one sugar, glucose, rhamnose, or galactose. Both flavonol and flavones are synthesized by the host plant and

are later metabolized or absorbed by the insect. Further research on this topic will benefit entomophagy as additional health benefits related to phenolic compounds could be obtained from insect consumption. In the following section, potential bioactivity of insect phenolics will be reviewed.

2.4 Potential bioactivity of insect phenolics

Phenolic compounds are known to exert diverse bioactivities linked to chronic diseases such as antioxidant, anti-inflammatory, and anticancer, among others. Also, antimicrobial bioactivity of plant phenolic compounds has been extensively studied against several pathogenic and non-pathogenic microorganisms as a result of the increasing concern regarding microbial resistance to conventional antibiotic treatments, and the interest in developing clean-label food preservatives that will prevent the use of synthetic compounds in the food industry (Cushnie & Lamb, 2005; Daglia, 2012; Farhadi et al., 2019). Currently, insect phenolics have only been assayed for antioxidant bioactivity. However, their overall bioactivity towards oxidation, inflammation, hypertensive, and glycaemic inhibition is widely attributed to protein and peptide fractions in insects such as tropical banded crickets (*Gryllodes sigillatus*), mealworm (*Tenebrio molitor*) and desert locusts (*Schistocerca gregaria*) (Hall et al., 2018; Hall & Liceaga, 2020; Zielińska et al., 2017, 2018). Furthermore, the possibility for other bioactive activities are promising as some of the phenolic compounds found in insects such as kaempferol and quercetin, have shown bioactivity when extracted from plant sources.

2.4.1 Antioxidant activity

An antioxidant is a chemical compound that inhibits or slows down the oxidation of another compound (Shahidi & Ambigaipalan, 2015). As every oxidation reaction implies the corresponding reduction reaction, a compound that is capable of preventing oxidative processes can be considered a reductant (Craft et al., 2012). Nonetheless, a reductant is not always an antioxidant as the latter refers to biological systems. Antioxidants can be classified according to their mode of action as ‘primary antioxidants’, when they actively inhibit oxidation reactions by the hydrogen-atom transfer mechanism or the single electron transfer mechanism or, ‘secondary antioxidants’ when they prevent oxidation through indirect reactions by chelating a metal atom

that serves as a catalyst of the oxidation or when they operate as oxygen scavengers (Craft et al., 2012). Phenolic compounds are considered primary antioxidants as they are able to neutralize free radicals, decompose peroxide species or quench singlet and triplet oxygen species (Sang et al., 2002). They are also considered secondary antioxidants as they are able to bind to metal ions, depending on the number and location of hydroxyl groups in the molecule (Shahidi & Ambigaipalan, 2015). Kim and Lee (2004) studied the relationship between structure and antioxidant capacity as vitamin C equivalent antioxidant capacity (VCEAC) of a representative variety of polyphenols using the Trolox equivalent antioxidant capacity (TEAC) assay, showing that the antioxidant capacity of polyphenolics was superior to that of single phenolics. In the case of flavonoids, antioxidant capacity was related to the number of hydroxyl groups, the pattern of such hydroxylation (*ortho*-dihydroxy phenolic structure), and the fact that aglycones exhibit better VCEAC than their glycosylated structures because of the sugar masking effects that cause steric hindrance. The relevance of the study of antioxidant capacity relies on the natural generation of reactive oxygen species (ROS) in the human body that can affect as ROS can damage multiple components in the cell, including DNA, RNA, lipids, and proteins, impairing normal cell function (Craft et al., 2012). An excessive amount of ROS has been linked to inflammation, cardiovascular diseases, cancer, diabetes, and Alzheimer disease**,** among other major diseases (Craft et al., 2012). The most known ROS include superoxide radical anion (O2·-), hydroxyl radical (HO·), alkoxyl radicals (RO·) and peroxyl radicals (RO2·) (Amorati & Valgimigli, 2015).

In the case of insect phenolics, Liu et al. (2012) evaluated the antioxidant activity of phenolic ethanol extracts (EE) and water extracts (WE) obtained from dark black chafer beetle (*Holotrichia parallela*) by four *in vitro* assays using α-tocopherol and butylated hydroxytoluene (BHT) as antioxidant standards. In the inhibition of linoleic acid peroxidation, EE showed superior activity compared to BHT revealing that the compounds present in EE exert better peroxidation inhibition activity. On the other hand, WE showed higher reducing power than EE, but both extracts had less activity than the standards. In the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging activity assay, WE showed increased activity compared to EE and BHT but less than α-tocopherol. Finally, for the ferrous ion-chelating activity, both extracts showed improved chelation capacity versus EDTA (ethylenediaminetetraacetic acid) used as the positive control. Metal chelating activity is relevant as it relates to lipid peroxidation because iron is a catalyst for the oxidative reaction. Hence, high chelating capacity relates to oxidative prevention. As for the

phenolic characterization of the extracts, the authors identified and quantified catechin (7.66±0.05 mg/g extract) on the EE extract. Whether the presence of this compound in the insect body occurs as a result of the diet or the sclerotisation process remains unknown.

Most recently, del Hierro et al. (2020) quantified total phenolic compounds of house cricket (*Acheta domesticus*) and mealworm (*T. molitor*) extracts, obtained by using solvent extraction with ethanol and ethanol: water (1:1, v/v). Two extraction methods were used, ultrasound-assisted extraction (UAE) and pressurized liquid extraction (PLE). Total phenolic content was similar for both insects, appearing to be slightly higher for *A. domesticus* obtaining a value of 5.0 g GAE/100 g of extract using a UAE and ethanol:water (1:1, v/v), while a value of approximately 3.8 g GAE/100 g of extract was obtained for *T. molitor* under the same extraction conditions. *in vitro* antioxidant activity was evaluated with the DPPH assay, obtaining nearly 80% inhibition by the UAE method in the ethanol:water extracts for both insects. Lastly, Zhao et al. (2018) performed an *in vivo* study comparing the antioxidant effects of phenolic compounds from Kudingcha tea, a traditional plant tea and insect tea, made from the excrements of the moth (*Hydrillodes repugnalis*) larvae that develop in the fermented Kudingcha tea. The study used the *D*-galactose-induced oxidation mouse model and showed higher activity of insect tea phenolics compared to Kudingcha tea phenolics at more effectively reducing oxidative damage through the nitric oxide pathway. Although interesting antioxidant capacity has been found for insect phenolics, real human health benefits after consumption need to be determined in further studies. In addition, as insect-derived food products become more prominent in the market, the effect of typical processing methods on the phenolic compounds and their antioxidant activity needs to be considered. Insects are typically processed by methods such as roasting, freezing, extrusion, blanching, among others. The impact of these processes on the retention of insect phenolic compounds remains unknown. However, studies have shown that processes like blanching, roasting and extrusion do not significantly affect the total phenolic content present in fruits and plants, indicating that the functionality of these compounds can be maintained by optimizing processing time and temperature and should be considered when processing insects (Şensoy et al., 2006; Sikora et al., 2008; Takeoka et al., 2001).

2.4.2 Other potential bioactivities

Anti-inflammatory, anticancer and antimicrobial bioactivities are among the potential capacities that insect phenolics could exert. Further research is needed to obtain new insights on insect phenolics' potential as well as their impact in human health. Research in this topic appears promising as the same compounds that are present in insects, mainly quercetin, kaempferol and catechin, exert bioactivity when extracted from plant sources.

Anti-inflammatory capacity has been demonstrated by several phenolic compounds, mainly quercetin, kaempferol, (-)-epicatechin and luteolin that interfere at several stages in the pro-inflammatory pathway (Baek et al., 1999; Comalada et al., 2006; García-Mediavilla et al., 2007; Gil et al., 1994; Hämäläinen et al., 2007; Mahat et al., 2010; Schewe et al., 2002; Toker et al., 2004). In addition to anti-inflammatory bioactivity, anticancer activity appears promising as several studies have demonstrated the capacity of phenolic compounds such as kaempferol, quercetin and myricetin to act as anti-carcinogenic compounds due to their antioxidant and prooxidant capacities (Shahidi & Yeo, 2018). In one study, quercetin showed antitumor activity in prostate cancer cells involving the regulation of tumor suppressor genes and downregulation of oncogenes (Nair et al., 2004). In a different cell study, myricetin was reported to have significant anticancer activity in 1,2-dimethylhydrazine-induced carcinogenesis in colorectal cancer, exhibiting a decrease in the incidence of the number of tumors in rats, and an up-regulation in antioxidant enzymes such as catalase and glutathione peroxidase (Nirmala & Ramanathan, 2011).

Finally, among all phenolic compounds, flavonoids (mainly flavones, flavonols, flavane-3-ols, and chalcones) report the highest antimicrobial activity through diverse mechanisms attributed to their amphipathic features, given by hydrophobic substituents such as alkylamino chains and the presence of the heterocyclic ring (Plaper et al., 2003; Wang et al., 2017). Flavonols are reported to have remarkable action against Gram-positive bacteria such as *Staphylococcus aureus* and *Lactobacillus acidophilus* (Daglia, 2012). Quercetin and myricetin are reported to have superior antibacterial properties against *S. aureus,* methicillin resistant and *Staphylococcus epidermis* (Cushnie & Lamb, 2005; Farhadi et al., 2019). Given the antimicrobial bioactivity exerted by plant phenolics and the evidence of these phenolics being absorbed by insects, potential antimicrobial capacity in insect compounds can be achievable. So far, antimicrobial bioactivity has mainly been attributed to insect chitin and chitosan in various studies (Mohan et al., 2020).

2.5 Conclusion

Incorporation of phenolic compounds in insects due to their capacity to sequester and metabolize dietary phenolics has been proven through the characterization of these compounds in the insect's body compared to the host plant's phenolic composition. This important finding can have an impact in edible insect farms where a standardized diet is used.

In addition to dietary phenolics, insects are able to synthetize phenolic compounds and incorporate them to their cuticle through the sclerotisation process. Additional studies are required in order to quantify the contribution of these cuticle phenolics to the overall phenolic content of the insects. Further research on insect phenols may lead to the discovery of potential bioactivities. Anti-inflammatory, antioxidant, anticancer, and antimicrobial activities have been reported for the major phenolic compounds found in insects like kaempferol and quercetin when they are directly extracted from plants. It has been proven that these compounds are absorbed and/or metabolized by the insects through their diet. Antioxidant capacity of insect phenolics (absorbed from their diet) has shown promising results, suggesting that the phenolics obtained from insects' diet maintain their bioactive properties. Further studies in edible insects are needed to characterize their phenolic composition and evaluate their anti-inflammatory, antioxidant, anticancer, and antimicrobial properties. The potential bioactive properties of insect phenols suggest that entomophagy may contribute to the human diet with additional health benefits apart from their nutritional value. However, more studies are needed to determine if the quantity of these compounds is enough to have a real impact after consumption and determine the effects of insect processing methods on these compounds.

2.6 References

Amorati, R., & Valgimigli, L. (2015). Advantages and limitations of common testing methods for antioxidants. *Free Radical Research, 49*(5), 633-649.

Andersen, S., & Roepstorff, P. (1982). Sclerotization of insect cuticle—III. An unsaturated derivative of N-acetyldopamine and its role in sclerotization. *Insect Biochemistry, 12*(3), 269-276.

Andersen, S. O. (2010). Insect cuticular sclerotization: a review. *Insect biochemistry and molecular biology, 40*(3), 166-178.

Andersen, S. O., & Roepstorff, P. (2007). Aspects of cuticular sclerotization in the locust, *Scistocerca gregaria,* and the beetle, *Tenebrio molitor*. *Insect biochemistry and molecular biology, 37*(3), 223-234.

Baek, S.-H., Yun, S.-S., Kwon, T. K., Kim, J.-R., Chang, H.-W., Kwak, J.-Y., Kim, J.-H., & Kwun, K.-B. (1999). The effects of two new antagonists of secretory PLA2 on TNF, iNOS, and COX-2 expression in activated macrophages. *Shock (Augusta, Ga.), 12*(6), 473-478.

Burghardt, F., Fiedlert, K., & Proksch, P. (1997). Uptake of flavonoids from *Vicia villosa* (Fabaceae) by the lycaenid butterfly, *Polyommatus icarus* (Lepidoptera: Lycaenidae). *Biochemical Systematics and Ecology, 25*(6), 527-536.

Burghardt, F., Knüttel, H., Becker, M., & Fiedler, K. (2000). Flavonoid wing pigments increase attractiveness of female common blue (*Polyommatus icarus*) butterflies to mate-searching males. *Naturwissenschaften, 87*(7), 304-307.

Burghardt, F., Proksch, P., & Fiedler, K. (2001). Flavonoid sequestration by the common blue butterfly *Polyommatus icarus*: quantitative intraspecific variation in relation to larval hostplant, sex and body size. *Biochemical Systematics and Ecology, 29*(9), 875-889.

Carocho, M., & CFR Ferreira, I. (2013). The role of phenolic compounds in the fight against cancer–a review. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), 13*(8), 1236-1258.

Cheynier, V. (2012). Phenolic compounds: from plants to foods. *Phytochemistry Reviews, 11*(2), 153-177.

Comalada, M., Ballester, I., Bailon, E., Sierra, S., Xaus, J., Gálvez, J., de Medina, F. S., & Zarzuelo, A. (2006). Inhibition of pro-inflammatory markers in primary bone marrow-derived mouse macrophages by naturally occurring flavonoids: analysis of the structure–activity relationship. *Biochemical pharmacology, 72*(8), 1010-1021.

Craft, B. D., Kerrihard, A. L., Amarowicz, R., & Pegg, R. B. (2012). Phenol-based antioxidants and the in vitro methods used for their assessment. *Comprehensive Reviews in Food Science and Food Safety, 11*(2), 148-173.

Cushnie, T. T., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents, 26*(5), 343-356.

Daglia, M. (2012). Polyphenols as antimicrobial agents. *Current opinion in biotechnology, 23*(2), 174-181.

del Hierro, J. N., Gutiérrez-Docio, A., Otero, P., Reglero, G., & Martin, D. (2020). Characterization, antioxidant activity, and inhibitory effect on pancreatic lipase of extracts from the edible insects *Acheta domesticus* and *Tenebrio molitor*. *Food chemistry*, 125742.

Di Carlo, G., Mascolo, N., Izzo, A. A., & Capasso, F. (1999). Flavonoids: old and new aspects of a class of natural therapeutic drugs. *Life sciences, 65*(4), 337-353.

Farhadi, F., Khameneh, B., Iranshahi, M., & Iranshahy, M. (2019). Antibacterial activity of flavonoids and their structure–activity relationship: An update review. *Phytotherapy Research, 33*(1), 13-40.

Ferreres, F., Fernandes, F., Oliveira, J. M., Valentão, P., Pereira, J. A., & Andrade, P. B. (2009a). Metabolic profiling and biological capacity of *Pieris brassicae* fed with kale (*Brassica oleracea* L. var. acephala). *Food and Chemical Toxicology, 47*(6), 1209-1220.

Ferreres, F., Fernandes, F., Pereira, D. M., Pereira, J. A., Valentao, P., & Andrade, P. B. (2009b). Phenolics metabolism in insects: *Pieris brassicae− Brassica oleracea* var. *costata* ecological duo. *Journal of Agricultural and Food Chemistry, 57*(19), 9035-9043.

Ferreres, F., Valentão, P., Pereira, J. A., Bento, A., Noites, A., Seabra, R. M., & Andrade, P. B. (2008). HPLC-DAD-MS/MS-ESI screening of phenolic compounds in *Pieris brassicae* L. reared on *Brassica rapa* var. *rapa* L. *Journal of Agricultural and Food Chemistry, 56*(3), 844-853.

García-Mediavilla, V., Crespo, I., Collado, P. S., Esteller, A., Sánchez-Campos, S., Tuñón, M. J., & González-Gallego, J. (2007). The anti-inflammatory flavones quercetin and kaempferol cause inhibition of inducible nitric oxide synthase, cyclooxygenase-2 and reactive C-protein, and down-regulation of the nuclear factor kappaB pathway in Chang Liver cells. *European journal of pharmacology, 557*(2-3), 221-229.

Geuder, M., Wray, V., Fiedler, K., & Proksch, P. (1997). Sequestration and metabolism of host-plant flavonoids by the lycaenid butterfly *Polyommatus bellargus*. *Journal of Chemical Ecology, 23*(5), 1361-1372.

Gil, B., Sanz, M., Ferrandiz, M., Bustos, G., Paya, M., Gunasegaran, R., & Alcaraz, M. (1994). Accelerated communication: effects of flavonoids on Naja naja and human recombinant synovial phospholipases A2 and inflammatory responses in mice. *Life sciences, 54*(20), PL333-PL338.

Gomes, A., Fernandes, E., Lima, J. L., Mira, L., & Corvo, M. L. (2008). Molecular mechanisms of anti-inflammatory activity mediated by flavonoids. *Current Medicinal Chemistry, 15*(16), 1586-1605.

Hall, F., Johnson, P. E., & Liceaga, A. (2018). Effect of enzymatic hydrolysis on bioactive properties and allergenicity of cricket (Gryllodes sigillatus) protein. *Food chemistry, 262*, 39-47.

Hall, F., & Liceaga, A. (2020). Effect of microwave-assisted enzymatic hydrolysis of cricket (Gryllodes sigillatus) protein on ACE and DPP-IV inhibition and tropomyosin-IgG binding. *Journal of functional foods, 64*, 103634.

Hämäläinen, M., Nieminen, R., Vuorela, P., Heinonen, M., & Moilanen, E. (2007). Anti-inflammatory effects of flavonoids: genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-κB activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-κB activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. *Mediators of inflammation, 2007*.

Harborne, J. B. (1991). Chapter 11 - Flavonoid Pigments. In G. A. Rosenthal & M. R. Berenbaum (Eds.), *Herbivores: their Interactions with Secondary Plant Metabolites (Second Edition)* (pp. 389-429). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-597183-6.50016-4

Hirayama, C., Ono, H., Meng, Y., Shimada, T., & Daimon, T. (2013). Flavonoids from the cocoon of *Rondotia menciana*. *Phytochemistry, 94*, 108-112.

Hirayama, C., Ono, H., Tamura, Y., Konno, K., & Nakamura, M. (2008). Regioselective formation of quercetin 5-*O*-glucoside from orally administered quercetin in the silkworm, *Bombyx mori*. *Phytochemistry, 69*(5), 1141-1149.

Hirayama, C., Ono, H., Tamura, Y., & Nakamura, M. (2006). C-prolinylquercetins from the yellow cocoon shell of the silkworm, *Bombyx mori*. *Phytochemistry, 67*(6), 579-583.

Hopkins, T., & Ahmad, S. (1991). Flavonoid wing pigments in grasshoppers. *Experientia, 47*(10), 1089-1091.

Ignat, I., Volf, I., & Popa, V. I. (2011). A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food chemistry, 126*(4), 1821-1835.

Kim, D.-O., & Lee, C. Y. (2004). Comprehensive study on vitamin C equivalent antioxidant capacity (VCEAC) of various polyphenolics in scavenging a free radical and its structural relationship. *Critical reviews in food science and nutrition, 44*(4), 253-273.

Kurioka, A., & Yamazaki, M. (2002). Purification and identification of flavonoids from the yellow green cocoon shell (Sasamayu) of the silkworm, *Bombyx mori*. *Bioscience, biotechnology, and biochemistry, 66*(6), 1396-1399.

Lahtinen, M., Kapari, L., & Kenttä, J. (2006). Newly hatched neonate larvae can glycosylate: the fate of *Betula pubescens* bud flavonoids in first instar *Epirrita autumnata*. *Journal of Chemical Ecology, 32*(3), 537-546.

Liu, S., Sun, J., Yu, L., Zhang, C., Bi, J., Zhu, F., Qu, M., & Yang, Q. (2012). Antioxidant activity and phenolic compounds of *Holotrichia parallela* Motschulsky extracts. *Food chemistry, 134*(4), 1885-1891.

Mahat, M. Y. A., Kulkarni, N. M., Vishwakarma, S. L., Khan, F. R., Thippeswamy, B., Hebballi, V., Adhyapak, A. A., Benade, V. S., Ashfaque, S. M., & Tubachi, S. (2010). Modulation of the cyclooxygenase pathway via inhibition of nitric oxide production contributes to the anti-inflammatory activity of kaempferol. *European journal of pharmacology, 642*(1-3), 169-176.

Mohan, K., Ganesan, A. R., Muralisankar, T., Jayakumar, R., Sathishkumar, P., Uthayakumar, V., Chandirasekar, R., & Revathi, N. (2020). Recent insights into the extraction, characterization, and bioactivities of chitin and chitosan from insects. *Trends in Food Science & Technology*.

Morris, S. J., & Thomson, R. (1963). The flavonoid pigments of the marbled white butterfly (Melanargia galathea Seltz). *Journal of Insect Physiology, 9*(3), 391-399.

Moussian, B. (2010). Recent advances in understanding mechanisms of insect cuticle differentiation. *Insect biochemistry and molecular biology, 40*(5), 363-375.

Mun, S., Noh, M. Y., Dittmer, N. T., Muthukrishnan, S., Kramer, K. J., Kanost, M. R., & Arakane, Y. (2015). Cuticular protein with a low complexity sequence becomes cross-linked during insect cuticle sclerotization and is required for the adult molt. *Scientific reports, 5*, 10484.

Musundire, R., Zvidzai, C., & Chidewe, C. (2014a). Bio-Active Compounds Composition in Edible Stinkbugs Consumed in South-Eastern Districts of Zimbabwe. *International Journal of Biology, 6*. https://doi.org/10.5539/ijb.v6n3p36

Musundire, R., Zvidzai, C., Chidewe, C., Ngadze, R., Macheka, L., Manditsera, F., Mubaiwa, J., & Masheka, A. (2016). Nutritional and bioactive compounds composition of *Eulepida mashona,* an edible beetle in Zimbabwe. *Journal of Insects as Food and Feed, 2*(3), 179-187.

Musundire, R., Zvidzai, C., Chidewe, C., Samende, B., & Manditsera, F. (2014b). Nutrient and anti-nutrient composition of *Henicus whellani* (Orthoptera: Stenopelmatidae), an edible ground cricket, in south-eastern Zimbabwe. *International Journal of Tropical Insect Science, 34*(4), 223-231.

Nair, H. K., Rao, K. V., Aalinkeel, R., Mahajan, S., Chawda, R., & Schwartz, S. A. (2004). Inhibition of prostate cancer cell colony formation by the flavonoid quercetin correlates with modulation of specific regulatory genes. *Clin. Diagn. Lab. Immunol., 11*(1), 63-69.

Nirmala, P., & Ramanathan, M. (2011). Effect of myricetin on 1, 2 dimethylhydrazine induced rat colon carcinogenesis. *Journal of experimental therapeutics & oncology, 9*(2).

Plaper, A., Golob, M., Hafner, I., Oblak, M., Šolmajer, T., & Jerala, R. (2003). Characterization of quercetin binding site on DNA gyrase. *Biochemical and biophysical research communications, 306*(2), 530-536.

Quideau, S., Deffieux, D., Douat-Casassus, C., & Pouysegu, L. (2011). Plant polyphenols: chemical properties, biological activities, and synthesis. *Angewandte Chemie International Edition, 50*(3), 586-621.

Salminen, J.-P., Lahtinen, M., Lempa, K., Kapari, L., Haukioja, E., & Pihlaja, K. (2004). Metabolic modifications of birch leaf phenolics by an herbivorous insect: detoxification of flavonoid aglycones via glycosylation. *Zeitschrift für Naturforschung C, 59*(5-6), 437-444.

Salminen, J.-P., & Lempa, K. (2002). Effects of hydrolysable tannins on a herbivorous insect: fate of individual tannins in insect digestive tract. *Chemoecology, 12*(4), 203-211.

Sang, S., Lapsley, K., Jeong, W.-S., Lachance, P. A., Ho, C.-T., & Rosen, R. T. (2002). Antioxidative phenolic compounds isolated from almond skins (*Prunus amygdalus* Batsch). *Journal of Agricultural and Food Chemistry, 50*(8), 2459-2463.

Schewe, T., Kühn, H., & Sies, H. (2002). Flavonoids of cocoa inhibit recombinant human 5-lipoxygenase. *The Journal of nutrition, 132*(7), 1825-1829.

Schittko, U., Burghardt, F., Fiedler, K., Wray, V., & Proksch, P. (1999). Sequestration and distribution of flavonoids in the common blue butterfly *Polyommatus icarus* reared on *Trifolium repens*. *Phytochemistry, 51*(5), 609-614.

Şensoy, Í., Rosen, R. T., Ho, C.-T., & Karwe, M. V. (2006). Effect of processing on buckwheat phenolics and antioxidant activity. *Food chemistry, 99*(2), 388-393.

Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. *Journal of functional foods, 18*, 820-897.

Shahidi, F., & Yeo, J. (2018). Bioactivities of phenolics by focusing on suppression of chronic diseases: A review. *International journal of molecular sciences, 19*(6), 1573.

Sikora, E., Cieślik, E., Leszczyńska, T., Filipiak-Florkiewicz, A., & Pisulewski, P. M. (2008). The antioxidant activity of selected cruciferous vegetables subjected to aquathermal processing. *Food chemistry, 107*(1), 55-59.

Simmonds, M. S. (2003). Flavonoid–insect interactions: recent advances in our knowledge. *Phytochemistry, 64*(1), 21-30.

Sugumaran, M. (1998). Unified mechanism for sclerotization of insect cuticle. In *Advances in Insect Physiology* (Vol. 27, pp. 229-334). Elsevier.

Sugumaran, M. (2010). Chemistry of cuticular sclerotization. In *Advances in insect Physiology* (Vol. 39, pp. 151-209). Elsevier.

Takeoka, G. R., Dao, L., Flessa, S., Gillespie, D. M., Jewell, W. T., Huebner, B., Bertow, D., & Ebeler, S. E. (2001). Processing effects on lycopene content and antioxidant activity of tomatoes. *Journal of Agricultural and Food Chemistry, 49*(8), 3713-3717.

Tamura, Y., Nakajima, K.-i., Nagayasu, K.-i., & Takabayashi, C. (2002). Flavonoid 5-glucosides from the cocoon shell of the silkworm, *Bombyx mori*. *Phytochemistry, 59*(3), 275-278.

Thomson, D. L. (1926). The Pigments of Butterflies' Wings: Melanargia galatea. *Biochemical Journal, 20*(1), 73.

Toker, G., Küpeli, E., Memisoğlu, M., & Yesilada, E. (2004). Flavonoids with antinociceptive and anti-inflammatory activities from the leaves of *Tilia argentea* (silver linden). *Journal of ethnopharmacology, 95*(2-3), 393-397.

Wang, S., Wang, C., Gao, L., Cai, H., Zhou, Y., Yang, Y., Xu, C., Ding, W., Chen, J., & Muhammad, I. (2017). Rutin inhibits *Streptococcus suis* biofilm formation by affecting CPS biosynthesis. *Frontiers in pharmacology, 8*, 379.

Wiesen, B., Krug, E., Fiedler, K., Wray, V., & Proksch, P. (1994). Sequestration of host-plant-derived flavonoids by lycaenid butterfly *Polyommatus icarus*. *Journal of Chemical Ecology, 20*(10), 2523-2538.

Wilson, A. (1985). Flavonoid pigments of butterflies in the genus Melanargia. *Phytochemistry, 24*(8), 1685-1691.

Wilson, A. (1987). Flavonoid pigments in chalkhill blue (*Lysandra coridon Poda*) and other lycaenid butterflies. *Journal of Chemical Ecology, 13*(3), 473-493.

Zhao, X., Song, J.-L., Yi, R., Li, G., Sun, P., Park, K.-Y., & Suo, H. (2018). Comparison of antioxidative effects of Insect tea and its raw tea (Kuding tea) polyphenols in Kunming mice. *Molecules, 23*(1), 204.

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*(9), 970.

Zielińska, E., Baraniak, B., & Karaś, M. (2018). Identification of antioxidant and anti-inflammatory peptides obtained by simulated gastrointestinal digestion of three edible insects species (Gryllodes sigillatus, Tenebrio molitor, Schistocerca gragaria). *International Journal of Food Science & Technology, 53*(11), 2542-2551.

CHAPTER 3. TARGETED PHENOLIC CHARACTERIZATION AND ANTIOXIDANT BIOACTIVITY OF EXTRACTS FROM EDIBLE ACHETA DOMESTICUS

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3.1 Abstract

With entomophagy gaining popularity in the Western hemisphere as a solution for future food insecurity, research on alternative protein sources such as edible insects becomes relevant. Most of the research done in insects has been on their nutritional qualities, but little is known on bioactive compounds, such as polyphenols, that if present in insect, could provide additional benefits when the insect is consumed. In this study, methanolic extracts of house cricket (*Acheta domesticus*) from two farms and their corresponding feeds were obtained using a microwave-assisted extraction. Targeted phenolic characterization was accomplished through LC-MS/MS leading to the identification of 4-hydroxybenzoic acid, *p*-coumaric acid, ferulic acid and syringic acid as major phenolic compounds in both *A. domesticus* extracts. Furthermore, *in vitro* antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl radical cation (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical assays resulting in superior quenching activity of the *A. domesticus* extracts compared to the feeds. The discovery of phenolic compounds in *A. domesticus* implies the ability of this insect species to sequester and absorb dietary phenolics leading to possible added health benefits when consumed.

3.2 Introduction

Edible insects are becoming increasingly relevant as food insecurity and sustainability develop into a global concern. In 2013, the Food and Agricultural Organization (FAO), published the report 'Edible insects: future prospects for food and feed security' promoting insects as a key solution for food insecurity. This led to increase interest of academia to generate applicable knowledge regarding entomophagy (Payne et al., 2019; Sosa & Fogliano, 2017). However, it is important to denote that entomophagy has limited acceptance due to it being an unfamiliar practice

for almost all western cultures and even considered a cultural taboo, which has limited its incorporation in the current western diet (Van Huis, 2013). In this context and, to provide more scientific-based information, more research has been done on the nutritional benefits of edible insects (Roos & Van Huis, 2017). For example, insects have shown to contain high protein quantity and quality, even comparable to that of traditional livestock (Yi et al., 2013). In addition, insect production has shown to be more sustainable as insects have higher conversion efficiency, meaning that feed is more efficiently converted to body mass than conventional livestock (Halloran et al., 2016; Nakagaki & Defoliart, 1991; Poma et al., 2017; Rumpold & Schlüter, 2013). For example, house cricket (*Acheta domesticus*), is twice as efficient as chicken and 12 times more than efficient cattle (Van Huis, 2013). Moreover, studies on mealworm (*Tenebrio molitor),* house cricket *(Acheta domesticus*) and migratory locust (*Locusta migratoria*) evidenced that these three insects generated less greenhouse gas emissions such as CO_2 and CH_4 when compared to livestock and have reduced ammonia emissions that is closely related with nitrification and acidification of soils (Halloran et al., 2016; Oonincx et al., 2010; Rumpold & Schlüter, 2013).

Among the different farmed edible insects, *A. domesticus* has gained momentum in the Western hemisphere due to their complete nutritional profile including having high protein content (64.4-70.7 %, dry basis) (da Silva Lucas et al., 2020). In addition, these crickets can be a good source of vitamins and other micronutrients such as potassium, calcium, iron and magnesium (Calzada-Luna et al., 2020; Rumpold & Schlüter, 2013) . Aside from the nutritional qualities, the biological activity of insect extracts has been of interest in recent years. del Hierro et al. (2020) evaluated the *in vitro* antioxidant activity using the DPPH assay and the pancreatic lipase inhibitory capacity of *A. domesticus* and *T. molitor* extracts. Both insect extracts using ultrasound-assisted extraction (UAE) with ethanol:water had nearly 80 % inhibition of the DPPH radical. In addition, *T. molitor* extract was most effective in inhibiting pancreatic lipase activity. Nevertheless, information regarding bioactivity of *A. domesticus* extracts is scarce and, to date, there is no published data regarding other bioactive compounds such as polyphenols, present in *A. domesticus* or in other edible insects consumed in the Western hemisphere.

Phenolic compounds are secondary plant metabolites characterized by the presence of one or more aromatic rings possessing at least one hydroxyl group attached to the aromatic structure (Ignat et al., 2011). Extensive research shows that phenolic compounds have several bioactivities including antioxidant, anti-inflammatory, antimicrobial and anticancer (Carocho & CFR Ferreira,

2013; Di Carlo et al., 1999; Farhadi et al., 2019; Gomes et al., 2008) among others; and diets rich in phenolics are associated with human health benefits. Given the insects' herbivore feeding behavior and their complex interactions with plants, it would be expected that insects ingest plant phenolic compounds. In this matter, even though little research has been done, available data shows the ability of insects to sequester and metabolize plant phenolics from their diet (Nino et al., 2021). Burghardt et al. (2001) evaluated the flavonoid content of the common blue butterfly (*Polyommatus icarus*) identifying a selective absorption of quercetin and kaempferol from their host plants. Hirayama et al. (2013) also identified two flavonol glycosides of quercetin and kaempferol from the cocoon of the white caterpillar (*Rondotia menciana*) that were fed exclusively with mulberry (*Morus alba*) leaves. These glycosides were not identified in the host plant implying that the insect was able to metabolize dietary flavonoids for further incorporation in the cocoon.

Despite the relevance of *A. domesticus* in entomophagy and the extensive research done in the nutritional aspects of this insect, to our knowledge, no studies have been conducted on the characterization of phenolics or the role of the feed on the phenolic composition of farmed *A. domesticus*. This study aimed to first determine if the extracts from *A. domesticus* reared on two different commercial diets could contain phenolic compounds and whether these phenolics were related to their specific diets, and secondly, to determine if these extracts exhibit in vitro antioxidant activity.

Given the evidence of dietary phenolics in insects that has been reported in literature, as well as the evidence of in vitro antioxidant activity of insect extracts, our hypothesis is that *A. domesticus* extracts will have dietary phenolics and will exert in vitro antioxidant activity. Hence, the objective of the present study is to elucidate the phenolic composition of farmed *A. domesticus* consuming two different diets and evaluate their potential in vitro antioxidant activity.

3.3 Materials and Methods

3.3.1 Raw materials and chemicals

All materials and chemical reagents were purchased from Fisher Scientific (Waltham, MA, USA) and Sigma Aldrich (St. Louis, MO, USA), unless specified. The two *Acheta domesticus* samples (6-weeks old), organic *Acheta* and commercial *Acheta*, were obtained from two rearing farms (Aspire Food Group in Austin, TX and Ovipost, Inc. in Labelle, FL, USA), respectively.

Both cricket samples were shipped frozen and kept in a -20 ºC freezer until needed. The organic feed was obtained from Aspire Food Group (Austin, TX, USA). The main ingredient composition of the organic feed consisted of organic corn, organic soybean and organic alfalfa. The commercial feed used in the farm for the commercial *Acheta* was purchased from an online store (Mazuri® Exotic Animal Nutrition, www.mazuri.com). The main ingredient composition of the commercial feed consisted of dehydrated alfalfa meal, wheat middlings, ground corn, ground soybean hulls, dehulled soybean meal, ground wheat and dried beet pulp.

3.3.2 Preparation of cricket and feed extracts

Feed and *A. domesticus* samples were freeze-dried for 72 h and then milled into a powder before processing. Microwave-assisted extraction (MAE) was done as described previously by Liu et al. (2012) with a microwave accelerated reaction system (MDS, MARS-Xpress/230/60, CEM Corporation, USA). Briefly, 12 g of feed or cricket powder were suspended in 120 mL of petroleum ether while stirring for 30 min at room temperature. Then, the sample was microwaved at 900 W for 120 s at a controlled temperature of 40 ºC. The solvent was removed by filtration with a Whatman No. 1 filter, and the solid residue was collected for the next extraction step. The residue was then suspended in 120 mL of extraction solvent (methanol: d-water, 7:3 v/v), stirred for 30 min at room temperature and microwaved again at 900 W for 300 s at a controlled temperature of 50 ºC. The solvent was collected and separated from the solid residue by filtration using a Whatman No.1 filter, then concentrated in a rotary evaporator at 55 ºC, and finally freeze-dried for 48 h to obtain the extract. Freeze-dried extracts were stored at -85 ºC in sealed containers until further use. The extraction was done in duplicate for each sample.

3.3.3 Total phenolic content of extracts

The content of total phenolic compounds (TPC) of *A. domesticus* and feed extracts was determined in triplicate as described previously by Singleton and Rossi (1965) with modifications by Cuadrado-Silva et al. (2017). Briefly, 35 µL of extract (10 mg/mL) or standard was mixed with 150 µL of a 1 N Folin reagent and was left to react for 5 min in dark conditions at room temperature in a 96-well plate. Then, 115 µL of a 7.5 % (w/v) Na_2CO_3 solution was added, and the microplate was left in incubation at 40 ºC for 30 min in darkness. After allowing the microplate cool for 1 h,

the absorbance at 765 nm was measured. The results are expressed as g of gallic acid equivalents (GAE) per 100 g of extract using a standard curve of gallic acid (ranging from 40 to 240 µg/mL).

3.3.4 Phenolic composition by UPLC-MS/MS

A. domesticus and feed extracts (10 mg/mL) were dissolved in 0.1 % (w/v) formic acid in dd-water in preparation for solid-phase extraction (Oasis HLB extraction cartridges). The cartridges were activated using sequential passes of 1 % formic acid in methanol and 1 % formic acid in dd-water. Samples were loaded on the SPE cartridges, rinsed with 0.1 % formic acid prior to polyphenol elution with 0.1 % formic acid in methanol. Dried extracts were resolubilized in 0.5 mL of formic acid, dd-water, and methanol (0.1 : 49.9 : 50), filtered using a 0.45 µm PTFE filter, and analyzed by LC-MS/MS using a Waters Acquity I Class UPLC equipped with a XEVO TQD mass spectrometer (Waters, Milford, MA, USA). Phenolics were resolved with an Acquity UPLC BEH C18 (2.1 × 50 mm) column at a flow rate of 0.5 mL min^{-1} using a gradient elution profile based on a binary phase of 0.1 % formic acid in water (solvent A) and 0.1 % in acetonitrile (solvent B). Separation was achieved at 40 °C using the following gradient: initially 100 % A, 0–0.5 min 100–94 % A, 0.5–2 min 94–91 % A, 2–3 min 91–87 % A, 3–4.5 min 87–65 % A, 4.5–5.5 min 65–100 % A, 5.5–6 min 100 % A. Phenolic compounds were detected under negative mode electrospray ionization (ESI-) with the following conditions: desolvation temperature 600 °C, desolvation gas flow 650 L h^{-1}, capillary voltage 3 kV, cone voltage 32 V, and collision energy of 20 V. Multiple reaction monitoring (MRM) responses were used to quantify individual phenolic compounds.

3.3.5 In vitro antioxidant activity

Two *in vitro* assays were used to evaluate *A. domesticus* and feed extracts' antioxidant potential. Freeze-dried extracts were reconstituted in distilled water and four different concentrations (20, 10, 5 and 2.5 mg/mL for commercial *Acheta*, organic feed and commercial feed, and 5, 2.5, 1.25 and 0.625 mg/mL for organic *Acheta*) were tested. Trolox was used as a positive standard.

DPPH (2,2-diphenyl-1-picrylhydrazyl radical cation) radical-scavenging activity

DPPH was carried out as described by Reddivari et al. (2007) with some modifications. First, a stock solution was prepared by dissolving 24 mg of DPPH in 100 mL ethanol. The working solution was prepared by diluting the stock solution until absorbance of 1.1 at 515 nm. Then, 15 µL of sample (*A. domesticus* or feed extracts) was mixed with 285 µL of DPPH working solution in a 96-well microplate. After incubation for 2 h in dark conditions, the absorbance at 515 nm was measured using distilled water as the control. Determinations were made in triplicate. Percentage of DPPH inhibition was determined with the following equation [1]:

$$\%\ inhibition = \left(\frac{A_{Control} - A_{Sample}}{A_{Control}}\right) \times 100 \qquad [1]$$

IC_{50} values denote the concentration of sample required to scavenge 50 % of the radical compound and were determined by interpolation from linear regression analysis.

ABTS (2,2'-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid) radical-scavenging activity

ABTS assay was developed following the method described by Ketnawa and Liceaga (2017) with modifications. First, a 7 mM stock solution of ABTS radical was prepared in a 2.45 mM solution of Potassium persulfate and left for incubation at room temperature for 16 h. After the incubation period, the working solution was prepared by diluting the stock solution with distilled water until absorbance of 0.7 at 734 nm. Then, 10 µL of sample (*A. domesticus* or feed extracts) was mixed with 294 µL of ABTS working solution in a 96-well microplate and was left in incubation in dark conditions at 30 ºC for 10 min. Following the incubation period, the absorbance at 740 nm was measured using distilled water as a control. The IC_{50} values were determined using Eq. [1] and by interpolation from linear regression analysis. Determinations were done in triplicate.

3.3.6 Protein content

Total crude protein of the feed and *Acheta* samples was determined as N x 6.25 by the Kjeldahl method (AOAC methods 984.13 using a certified commercial laboratory (A&L Great Lakes Laboratories, Fort Wayne, IN).

3.3.7 Statistical analysis

All experiments and analyses were conducted in triplicate, unless otherwise indicated. Statistical analysis of observed differences among means was performed using one-way analysis of variance (ANOVA), followed by Tukey's pairwise comparison of means at a 5 % significance level with the statistical software Minitab 18® (State College, PA, USA).

3.4 Results and discussion

3.4.1 Extraction yields

The extract yields of *A. domesticus* and feed using microwave-assisted extraction (MAE) are given in Table 2. The extract yield of the organic *Acheta* and commercial *Acheta* extracts were 7.85 % and 8.42 %, respectively. In other insect species such as the rhinoceros beetle (*Allomyrina dichotoma*) the extraction yield with 80 % methanol was 0.70 % (Suh et al., 2010), much lower than the yields reported in the present study. In another study, the extraction yields of water and ethanol extracts of dark black chafer beetle (*Holotrichia parallela*) using MAE are reported as 25.03 % and 10.22 %, respectively (Liu et al. 2012). In this case, the *H. parallela* water extract had a higher yield in comparison to our *A. domesticus* extracts but the ethanol extract yield appears to have a similar value to the yields reported in this study using the same extraction method. The feed extracts had an extraction yield of 6.48 % for the organic feed and 7.83 % for the commercial feed, both similar to the *A. domesticus* yields. This similarity indicates that the extraction method can be used to obtain diverse compounds from plant as well as animal sources, since both types of samples had comparable yields. In addition, the microwave-assisted extraction method used in this study successfully extracted polyphenols allowing for the characterization of such compounds.

3.4.2 Total phenolic content of extracts

A significant difference was observed ($p<0.05$) for the total phenolic content of *A. domesticus* and feed extracts (Table 2). These values are in agreement with the values reported by del Hierro et al. (2020) for *A. domesticus* ethanol extracts obtained my means of ultrasound-assisted extraction (UAE) and pressurized liquid extraction (PLE), where the total phenolic content was within a range of 0.3-5.0 g GAE/100 g of sample. Even though scarce information is available for edible insects, total phenolic content has been reported in recent studies. Musundire et al.

(2014a) reported a value of 3.6 g GAE/100 g for unprocessed edible stinkbugs (*Encosternum delegorguei*), where the authors also observed a decrease in total phenolics of the insect after applying traditional cooking techniques used in Zimbabwe. In a similar study, the total phenolic content of unprocessed edible beetle (*Eulepida mashona*) resulted in 0.08 g GAE/100 g of sample (Musundire et al., 2016). In another study, the total phenolic content of the edible ground cricket (*Henicus whellani*) was 0.77 g GAE/100 g (Musundire et al., 2014b), which is lower than the values reported in this study for *A. domesticus.* Additionally, Liu et al. (2012) reported 5 g GAE/ 100 g of sample for MAE extracts of *H. parallela*. This value is close to the phenolic content reported in this study for the *A. domesticus* extracts obtained using a similar extraction method (MAE). Likewise to the *A. domesticus* extracts, the feed extracts were significantly different ($p < 0.05$) from each other, with the commercial feed having a higher value than the organic feed. Overall, the feed extracts had a lower total phenolic content in comparison to the *A. domesticus* extracts. Given that the samples (insect and feed) are of different composition and the *A. domesticus* extracts represent a more complex matrix compared to the feeds, the higher total phenolic content in *A. domesticus* cannot be attributed only to the phenolic compounds present in the sample, but also to other components that can react with the Folin-Ciocalteu reagent. For example, the cricket extracts could contain unsaturated fatty acids, vitamins, and free amino acids that have shown reactivity with the Folin-Ciocalteu reagent in previous studies (Everette et al., 2010). Despite the fact that the Folin-Ciocalteu assay is a well-known method to quantify phenolic compounds in diverse samples, the reaction mechanism is not specific for phenolics (Magalhães et al., 2010). For example, other reducing compounds such as ascorbic acid and select amino acids that could contribute to the total phenolic content, leading to an over-estimation of the phenolic compounds in the samples.

The crude protein was determined for each sample (Table 2). Even though no direct correlation can be made between protein content and total phenolic content values, the relationship between these two variables is still observable. Both *A. domesticus* samples with higher protein content resulted in higher total phenolic content in comparison with both feeds that had less protein and lower total phenolic content. Hence the need to utilize more accurate and sensible methods like the LC-MS/MS technique used in this study, to assess the phenolic content of the extracts (Liu et al., 2012; Suh et al., 2010).

Table 2. Total Phenolic Content (TPC) and crude protein of *A. domesticus* and feed extracts.

Extracts	Organic *Acheta*	Commercial *Acheta*	Organic Feed	Commercial Feed
TPC (g GAE/ 100g extract)	2.1 ± 0.05^{a}	1.9 ± 0.04^{b}	1.2 ± 0.08^{x}	1.5 ± 0.08^{y}
Crude protein (g/100g)	45.13	43.88	9.88	13.94
Yield (%)	7.9 ± 0.27	8.4 ± 0.12	6.5 ± 0.02	7.8 ± 0.19

Total phenolic content (TPC) values (g GAE/100g extract) are expressed as the mean ± standard deviation (n=6). A comparison of TPC was made between the organic *Acheta* and commercial *Acheta*, and between organic feed and commercial feed, respectively. Different superscript letters indicate significant differences ($p< 0.05$) between samples. Protein content values are expressed as g/100g of sample and were determined by a certified commercial laboratory. No SD values are shown for crude protein as only one analysis was made due to the small quantity of sample.

3.4.3 Phenolic composition of extracts using LC-MS

UPLC-MS/MS analysis allowed for the identification of 17 individual phenolic compounds in both feeds (organic and commercial), 13 were identified in the organic *Acheta* extract, and 11 in the commercial *Acheta* extract (Table 3). Major compounds identified in both *Acheta* extracts correspond to 4-hydroxybenzoic acid, *p*-coumaric acid, ferulic acid and syringic acid. A significant difference ($p<0.05$) was observed in the concentration of *p*-coumaric acid and syringic acid, where the organic *Acheta* appears to have a higher concentration of both compounds. Even though the *Acheta domesticus* characterization shows very low concentrations of phenolics, these results can still be associated with their corresponding feeds. Similar to the *Acheta* extracts, the same four phenolic acids constitute the major phenolic compounds identified in both feeds. No significant difference ($p>0.05$) was observed between the feed extracts except for ferulic acid and chlorogenic acid, where the commercial feed had a higher concentration (Table 3). Given the presence of the same phenolic acids, and particularly that the same major compounds were found in both sets of samples (*Acheta* and feed extracts), it is possible that farmed *A. domesticus* are able to absorb and sequester dietary phenolic acids from their feed. Confirmation of phenolic compounds in insects has been previously reported for a variety of species, mainly Lepidopterans (e.g., butterflies and

moths), where the main assumption is that these compounds are directly obtained from the insects' diet (Burghardt et al., 1997; Burghardt et al., 2001; Wiesen et al., 1994). This hypothesis has been proven as some of the phenolics detected in the insect are also found in the host plants in which the insects are reared that constitute their primary feeding source (Geuder et al., 1997; Hirayama et al., 2013; Schittko et al., 1999). Evidence of absorption of dietary phenolic acids was reported previously in the literature. For example, Ferreres et al. (2008) reported the presence of phenolic acids, ferulic, sinapic and *p*-coumaric, in extracts of larvae of the large white butterfly (*Pieris brassicae*) reared on turnip (*Brassica rapa* var. *rapa* L*).* In a follow-up study, ferulic and sinapic acids were also found in this insect when reared on the tronchuda cabbage (*Brassica oleracea* var. *costata*) (Ferreres et al., 2009b). As reported, chlorogenic acid was detected in both feeds, being present at higher concentrations in the commercial feed. However, this compound was not found in the *Acheta* extracts. This could imply that *A. domesticus* is not able to absorb this compound or that it is metabolized and hence, not detected. Evidence of excretion of phenolic compounds without being absorbed by the insect has been reported previously (Burghardt et al., 1997; Ferreres et al., 2009b). Further studies on the metabolism of plant phenolics by *A. domesticus* are needed to have more clarity of the fate on these compounds when ingested by the insect.

This study aimed to analyze if *A. domesticus* would be able to absorb dietary phenolics and if so, how the composition of the feed influences the phenolic composition of *A. domesticus*. The main ingredient composition of the commercial feed consisted of dehydrated alfalfa meal, wheat middlings, ground corn, ground soybean hulls, dehulled soybean meal, ground wheat, and dried beet pulp. In contrast, the organic feed consisted mainly of organic corn, organic soybean and organic alfalfa. Nevertheless, no difference in the phenolic composition of the feed was observed for the 17 individual phenolic compounds identified in this study (Table 3). Hence, no difference in the phenolic composition of the insects was expected.

The phenolic characterization showed a very low concentration of phenolic compounds for the insects as well as for the feed extracts. This might be attributed to the processing method of the feed. Both feeds require milling and drying processes that have positive as well as detrimental effects on the phenolic content of the raw plant material (Cheynier, 2012; Ifie & Marshall, 2018). It is possible that the heating and grinding conditions utilized for the feed production, as well as the storage conditions and age of the final feed product, resulted in a decrease of the overall phenolic content on the feed, leading to low quantities of the phenolic compounds and, low

quantities in the insects. In contrast to the low quantities detected for the feed and *Acheta* extracts in this study (ng/10mg), in other studies the concentrations of the main phenolic compounds are in µg/g or mg/g (Schittko et al., 1999; Wiesen et al., 1994). This difference might be related to the insect's diet as in previous studies the insects were reared in their host plants, fresh turnip and cabbage, which would have a higher concentration of phenolic compounds. Furthermore, the use of a processed diet instead of a fresh-plant diet could have an impact on the absorption of phenolics by the insect. Burghardt et al. (2001) observed that the absorption of phenolics was higher when *P. icarus* was fed natural host plants, compared to an experimental diet. Given that the farmed *A. domesticus* used for this study were fed with a processed diet instead of a fresh plant diet, the absorption of phenolics could have been affected.

As far as we know, this is the first time that the phenolic compounds in farmed *A. domesticus* fed two different processed and/or commercial diets have been reported. Because the insects used for this study were not starved before harvesting, it is unclear if the phenolic compounds were effectively absorbed or if the insect's excrement composition is contributing to the overall phenolic content. Follow-up studies are necessary to confirm dietary phenolic absorption as well as the impact of sclerotization-derived phenolics in the overall phenolic composition of the insect. Nonetheless, this approach allowed for the characterization in real conditions, of the phenolic composition in farmed *A. domesticus* with the corresponding feed used by most edible insect farms in the USA. The results obtained in this study are of great interest since the discovery of these potential bioactive compounds in *A. domesticus* could potentially lead to added health benefits related to their consumption that were not previously contemplated. Further studies, especially in bioavailability and bioaccesibility of these compounds could increase the interest on entomophagy, as this knowledge would represent an added value to insect consumption in addition to the nutritional characteristics (e.g protein content).

Table 3. Characterization of targeted of targeted phenolic compounds in *A. domesticus* and feed extracts by liquid chromatography coupled with electrospray-ionization triple quadrupole mass spectrometry (LC-ESI-QqQ-MS/MS).

Target compound	RT (min)	Molecular weight	MRM Transition	Phenolic compounds (ng/10mg)			
				Organic Acheta	Commercial Acheta	Organic Feed	Commercial Feed
Phenolic acids							
Quinic acid	0.34	192	191>85	1.6 ± 0.9^{a}	4.0 ± 2.2^{a}	6.5 ± 0.9^{x}	6.0 ± 1.5^{x}
Gallic acid	0.64	170	169>125	1.4 ± 0.1^{a}	0.5 ± 0.2^{b}	6.3 ± 0.7^{x}	9.2 ± 0.9^{y}
4-hydroxybenzoic acid	1.28	138	137>93	29.4 ± 3.3^{a}	20.6 ± 2.9^{a}	79.9 ± 4.6^{x}	70.3 ± 3.9^{x}
Chlorogenic Acid	1.53	354	353>191	3.1 ± 0.7^{a}	1.5 ± 0.7^{a}	11.8 ± 1.3^{x}	104.0 ± 13.2^{y}
Caffeic acid	1.69	180	179>135	1.8 ± 0.3^{a}	0.8 ± 0.1^{b}	16.7 ± 1.6^{x}	35.8 ± 4.5^{y}
Syringic acid	1.98	198	197>167	13.8 ± 3.5^{a}	4.7 ± 1.0^{b}	127.3 ± 14.6^{x}	132.8 ± 15.3^{x}
p-coumaric acid	2.5	164	163>119	7.0 ± 1.3^{a}	5.8 ± 0.7^{b}	115.0 ± 9.1^{x}	126.5 ± 15.7^{x}
Ferulic acid	3.08	194	193>134	9.9 ± 1.2^{a}	12.9 ± 1.6^{a}	95.0 ± 5.4^{x}	144.2 ± 9.1^{y}
Sinapic acid	3.24	224	223>208	3.0 ± 0.2^{a}	1.8 ± 0.2^{b}	33.1 ± 3.7^{x}	20.6 ± 2.3^{y}
2-hydroxybenzoic acid	3.59	138	137>65	1.9 ± 0.2	n.d	20.0 ± 1.5^{x}	12.8 ± 1.0^{y}
Flavonoids							
Quercetin-3-glucoside	3.41	464	463>300	n.d	n.d	7.2 ± 0.8^{x}	88.8 ± 4.7^{y}
Quercetin-3-rutinoside	3.48	610	609>300	n.d	n.d	1.5 ± 0.1^{x}	2.3 ± 0.2^{y}
Kaempferol-3-glucoside	3.71	448	447>284	n.d	n.d	6.8 ± 0.8^{x}	8.2 ± 0.5^{x}
Daidzein	3.73	254	253>91	0.1 ± 0.0	n.d	7.4 ± 0.1^{x}	5.6 ± 0.2^{y}
Quercetin	4.28	302	301>151	1.3 ± 0.6	n.d	6.3 ± 0.6^{x}	14.9 ± 0.9^{y}
Naringenin	4.54	272	271>151	1.0 ± 0.1^{a}	0.7 ± 0.1^{b}	2.6 ± 0.2^{x}	3.1 ± 0.3^{x}
Apigenin	4.57	270	269>117	3.0 ± 0.2^{a}	1.9 ± 0.0^{b}	26.4 ± 2.4^{x}	13.4 ± 0.4^{y}

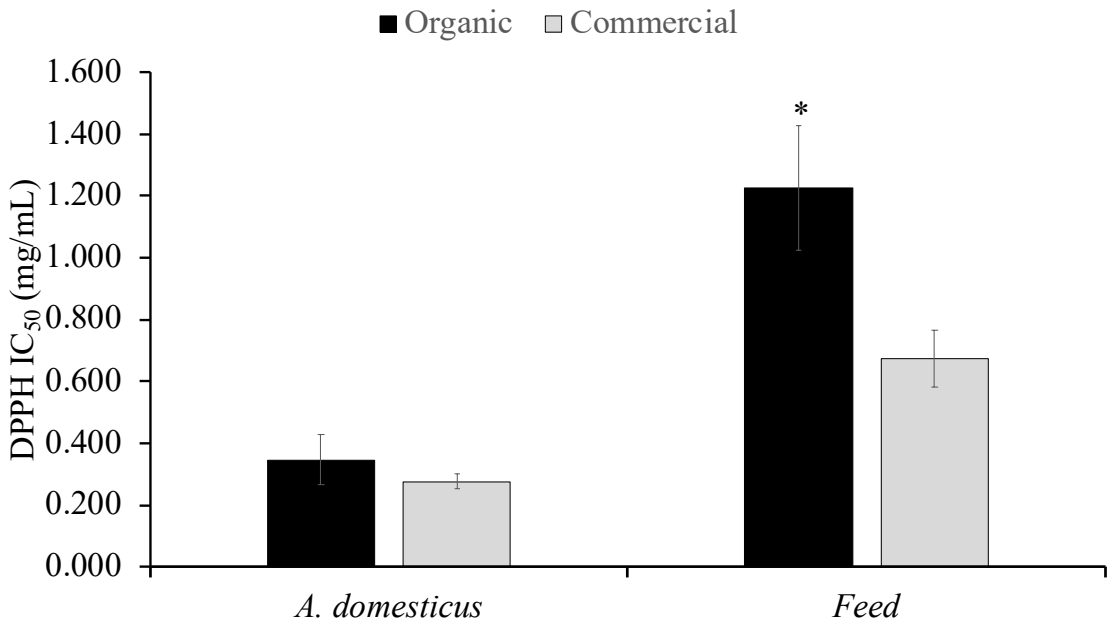
Values are expressed as the mean ± standard error (n=6). Each phenolic compound was analyzed separately. Comparison was made between the organic *Acheta* and commercial *Acheta* and between the organic feed and commercial feed, respectively. Different superscript letters (a, b and x, y) indicate significant differences (p-value < 0.05) between sample groups (*Acheta* and feed, respectively); "n.d" implies the compound was not detected.

3.4.4 *In vitro* antioxidant activity

The potential antioxidant activity of the extracts was evaluated by their ability to inhibit the $DPPH^{\cdot}$ and the $ABTS^{\cdot+}$ radicals. Although for both assays the antioxidant compound can exert its quenching activity by the hydrogen-atom transfer mechanism (HAT) or the single electron transfer mechanism (SET), SET appears to be the main mechanism occurring in the DPPH assay (Craft et al., 2012; Shahidi & Zhong, 2015). As the quenching ability of the antioxidant occurs through the same mechanisms in both tests, a comparison among them is acceptable. The antioxidant activity against the DPPH radical was superior for both *Acheta* extracts having IC_{50} values of 0.346 mg/mL and 0.275 mg/mL for the organic *Acheta* and commercial *Acheta*, respectively (Figure 3a). The same result was obtained in the ABTS assay, where the *Acheta* extracts exhibited higher scavenging potential against this radical when compared to the feeds (Figure 3b), supporting the capacity of constituents of the extracts to act as primary antioxidants. The results obtained for the feed extracts showed a higher ($p<0.05$) quenching ability by the commercial feed (DPPH IC_{50}= 0.674 and ABTS IC_{50}= 0.146) compared to the organic feed (DPPH IC_{50}= 1.228 and ABTS IC_{50}= 0.179).

Antioxidant potential of insect extracts has been previously evaluated using the DPPH radical assay showing promising antioxidant activity. Suh et al. (2010) evaluated the antioxidant activity of Japanese rhinoceros beetle (*A. dichotoma)* extracts reporting moderate scavenging action depending on the extraction solvent, with the lowest IC_{50} (0.119 mg/mL) being for the methanolic extract. Furthermore, aqueous MAE of *H. parallela* exhibited relevant antioxidant activity reporting an IC_{50} of 1.45 mg/mL (Liu et al., 2012). The IC_{50} values obtained for *A. domesticus* in this study are similar to those reported for *A. dichotoma* that was extracted with a similar solvent. However, they differ from the values reported for *H. parallela* using a similar extraction method. The similarity in the IC_{50} values could imply similar antioxidant activity with the *A. dichotoma* extracts, while the lower value compared to *H. parallela* could indicate the better antioxidant activity of the *Acheta* extracts.

a)



b)

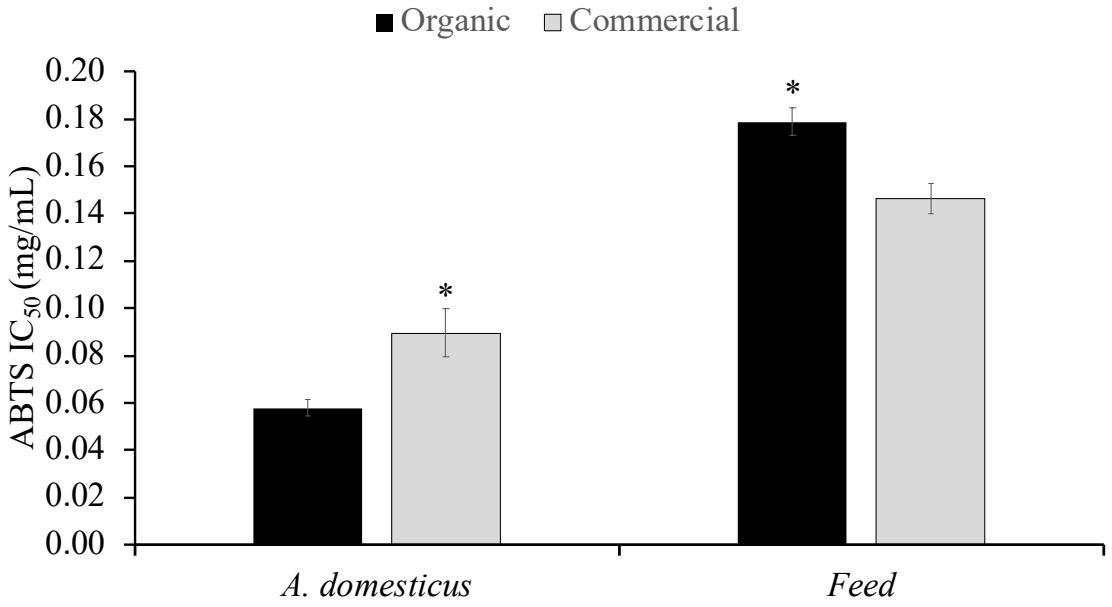


Figure 3. Antioxidant activity assays of extracts of *A. domesticus* (organic *Acheta* and commercial *Acheta*) and feed (organic feed and commercial feed): (a) DPPH radical scavenging activity (IC50 value, mg extract/mL); (b) ABTS radical scavenging activity (IC50 value, mg extract/mL). Asterisk (*) indicates significant difference ($p<0.05$) between *A. domesticus* and feed extracts, respectively.

The antioxidant activity of insect extracts has been previously attributed to the presence of phenolic compounds (mainly flavonoids) that have been long recognized to be potent antioxidants. Their antioxidant activity is due the ability to neutralize free radicals, decompose peroxide species and quench singlet and triplet oxygen (Sang et al., 2002), or as secondary antioxidants they can bind metal ions (Craft et al., 2012). Nonetheless, it is also acknowledged that the presence of bioactive peptides, proteins and free amino acids may also contribute to the antioxidant activity of the extracts (Suh et al., 2010). In addition, a correlation between total phenolic content and DPPH scavenging activity has been recently reported for *T. molitor* and *A. domesticus* extracts, where those with the higher total phenolic content had higher DPPH inhibition (del Hierro et al., 2020). As discussed previously, given that the Folin-Ciocalteu reagent is not selective to phenolics and uses tungsten ion to oxidize other compounds like amino acid residues, it is likely that the observed bioactivity is a result of a group of bioactive molecules and not only phenolic compounds (Suh et al., 2010).

In this study, due to the low quantities of phenolic compounds detected in the *Acheta* extracts, the observed antioxidant bioactivity cannot be attributed only to the phenolic compounds that were characterized, but to a synergistic interaction between these compounds and proteins mainly, which are components found in the extracts (Table 1). Further studies on the characterization of other possible phytochemicals that could be present in the extracts, as well as peptides could lead to a better understanding of the bioactive potential of the insect extracts. Although *in vitro* antioxidant activity serves as evidence of potential bioactivity of the extracts, this information is not sufficient to evaluate the real impact on human health as evaluation of bioaccesibility and bioavailability are needed. Future studies evaluating these parameters like the use of simulated gastrointestinal digestion coupled with cell culture models (e.g Caco-2-cells) to evaluate bioaccessibility and *in vivo* models to evaluate bioavailability (fraction of the bioactive compounds that is absorbed and that reaches the systemic circulation) could confirm the potential antioxidant benefits of insect consumption (Ferreira et al., 2017; Santos et al., 2019).

3.5 Conclusion

The data presented in this study confirms for the first time the presence of dietary phenolics in farmed *Acheta domesticus*, suggesting that *A. domesticus* may be able to absorb or sequester dietary phenolics. The following phenolic acids, 4-hydroxybenzoic acid, *p*-coumaric acid, ferulic

acid and syringic acid were found to be the major phenolic compounds present in *A. domesticus* and feed extracts. In addition, microwave-assisted extracts of *A. domesticus* exerted *in vitro* antioxidant activity, higher than that exhibited by their plant-based feed extracts. Phenolics as well as proteins may contribute to the antioxidant activity of the extracts. The results of this study suggest that farmed *A. domesticus* could potentially provide additional health benefits when consumed. Nonetheless, due to the low quantities of these compounds, further studies regarding bioavailability and bioaccesibility are needed in order to confirm the potential health benefits.

3.6 References

Burghardt, F., Fiedlert, K., & Proksch, P. (1997). Uptake of flavonoids from *Vicia villosa* (Fabaceae) by the lycaenid butterfly, *Polyommatus icarus* (Lepidoptera: Lycaenidae). *Biochemical Systematics and Ecology, 25*(6), 527-536.

Burghardt, F., Proksch, P., & Fiedler, K. (2001). Flavonoid sequestration by the common blue butterfly *Polyommatus icarus*: quantitative intraspecific variation in relation to larval hostplant, sex and body size. *Biochemical Systematics and Ecology, 29*(9), 875-889.

Calzada-Luna, G., San Martin-Gonzalez, F., Mauer, L., & Liceaga, A. M. (2020). Cricket (Acheta domesticus) Protein Hydrolysates Impact on the Physicochemical, Structural and Sensory properties of Tortillas and Tortilla chips. *Journal of Insects as Food and Feed, In press*.

Carocho, M., & CFR Ferreira, I. (2013). The role of phenolic compounds in the fight against cancer–a review. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents), 13*(8), 1236-1258.

Cheynier, V. (2012). Phenolic compounds: from plants to foods. *Phytochemistry Reviews, 11*(2), 153-177.

Craft, B. D., Kerrihard, A. L., Amarowicz, R., & Pegg, R. B. (2012). Phenol-based antioxidants and the in vitro methods used for their assessment. *Comprehensive Reviews in Food Science and Food Safety, 11*(2), 148-173.

Cuadrado-Silva, C., Pozo-Bayón, M., & Osorio, C. (2017). Targeted metabolomic analysis of polyphenols with antioxidant activity in sour guava (Psidium friedrichsthalianum Nied.) fruit. *Molecules, 22*(1), 11.

da Silva Lucas, A. J., de Oliveira, L. M., da Rocha, M., & Prentice, C. (2020). Edible insects: An alternative of nutritional, functional and bioactive compounds. *Food chemistry, 311*, 126022.

del Hierro, J. N., Gutiérrez-Docio, A., Otero, P., Reglero, G., & Martin, D. (2020). Characterization, antioxidant activity, and inhibitory effect on pancreatic lipase of extracts from the edible insects *Acheta domesticus* and *Tenebrio molitor*. *Food chemistry*, 125742.

Di Carlo, G., Mascolo, N., Izzo, A. A., & Capasso, F. (1999). Flavonoids: old and new aspects of a class of natural therapeutic drugs. *Life sciences, 65*(4), 337-353.

Everette, J. D., Bryant, Q. M., Green, A. M., Abbey, Y. A., Wangila, G. W., & Walker, R. B. (2010). Thorough study of reactivity of various compound classes toward the Folin− Ciocalteu reagent. *Journal of Agricultural and Food Chemistry, 58*(14), 8139-8144.

Farhadi, F., Khameneh, B., Iranshahi, M., & Iranshahy, M. (2019). Antibacterial activity of flavonoids and their structure–activity relationship: An update review. *Phytotherapy Research, 33*(1), 13-40.

Ferreira, I. C., Martins, N., & Barros, L. (2017). Phenolic compounds and its bioavailability: In vitro bioactive compounds or health promoters? In *Advances in food and nutrition research* (Vol. 82, pp. 1-44). Elsevier.

Ferreres, F., Fernandes, F., Pereira, D. M., Pereira, J. A., Valentao, P., & Andrade, P. B. (2009). Phenolics metabolism in insects: *Pieris brassicae*− *Brassica oleracea* var. *costata* ecological duo. *Journal of Agricultural and Food Chemistry, 57*(19), 9035-9043.

Ferreres, F., Valentão, P., Pereira, J. A., Bento, A., Noites, A., Seabra, R. M., & Andrade, P. B. (2008). HPLC-DAD-MS/MS-ESI screening of phenolic compounds in *Pieris brassicae* L. reared on *Brassica rapa* var. *rapa* L. *Journal of Agricultural and Food Chemistry, 56*(3), 844-853.

Geuder, M., Wray, V., Fiedler, K., & Proksch, P. (1997). Sequestration and metabolism of host-plant flavonoids by the lycaenid butterfly *Polyommatus bellargus*. *Journal of Chemical Ecology, 23*(5), 1361-1372.

Gomes, A., Fernandes, E., Lima, J. L., Mira, L., & Corvo, M. L. (2008). Molecular mechanisms of anti-inflammatory activity mediated by flavonoids. *Current Medicinal Chemistry, 15*(16), 1586-1605.

Halloran, A., Roos, N., Eilenberg, J., Cerutti, A., & Bruun, S. (2016). Life cycle assessment of edible insects for food protein: a review. *Agronomy for Sustainable Development, 36*(4), 57.

Hirayama, C., Ono, H., Meng, Y., Shimada, T., & Daimon, T. (2013). Flavonoids from the cocoon of *Rondotia menciana*. *Phytochemistry, 94*, 108-112.

Ifie, I., & Marshall, L. J. (2018). Food processing and its impact on phenolic constituents in food. *Cogent Food & Agriculture, 4*(1), 1507782.

Ignat, I., Volf, I., & Popa, V. I. (2011). A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. *Food chemistry, 126*(4), 1821-1835.

Ketnawa, S., & Liceaga, A. M. (2017). Effect of microwave treatments on antioxidant activity and antigenicity of fish frame protein hydrolysates. *Food and bioprocess technology, 10*(3), 582-591.

Liu, S., Sun, J., Yu, L., Zhang, C., Bi, J., Zhu, F., Qu, M., & Yang, Q. (2012). Antioxidant activity and phenolic compounds of *Holotrichia parallela* Motschulsky extracts. *Food chemistry, 134*(4), 1885-1891.

Magalhães, L. M., Santos, F., Segundo, M. A., Reis, S., & Lima, J. L. (2010). Rapid microplate high-throughput methodology for assessment of Folin-Ciocalteu reducing capacity. *Talanta, 83*(2), 441-447.

Musundire, R., Zvidzai, C., & Chidewe, C. (2014a). Bio-Active Compounds Composition in Edible Stinkbugs Consumed in South-Eastern Districts of Zimbabwe. *International Journal of Biology, 6*. https://doi.org/10.5539/ijb.v6n3p36

Musundire, R., Zvidzai, C., Chidewe, C., Ngadze, R., Macheka, L., Manditsera, F., Mubaiwa, J., & Masheka, A. (2016). Nutritional and bioactive compounds composition of *Eulepida mashona,* an edible beetle in Zimbabwe. *Journal of Insects as Food and Feed, 2*(3), 179-187.

Musundire, R., Zvidzai, C., Chidewe, C., Samende, B., & Manditsera, F. (2014b). Nutrient and anti-nutrient composition of *Henicus whellani* (Orthoptera: Stenopelmatidae), an edible ground cricket, in south-eastern Zimbabwe. *International Journal of Tropical Insect Science, 34*(4), 223-231.

Nakagaki, B. J., & Defoliart, G. R. (1991). Comparison of diets for mass-rearing Acheta domesticus (Orthoptera: Gryllidae) as a novelty food, and comparison of food conversion efficiency with values reported for livestock. *Journal of Economic Entomology, 84*(3), 891-896.

Nino, M., Reddivari, L., Osorio, C., Kaplan, I., & Liceaga, A. (2021). Insects as a source of phenolic compounds and potential health benefits. *Journal of Insects as Food and Feed*, 1-12.

Oonincx, D. G., Van Itterbeeck, J., Heetkamp, M. J., Van Den Brand, H., Van Loon, J. J., & Van Huis, A. (2010). An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. *PloS one, 5*(12), e14445.

Payne, C., Megido, R. C., Dobermann, D., Frédéric, F., Shockley, M., & Sogari, G. (2019). Insects as Food in the Global North–The Evolution of the Entomophagy Movement. In *Edible Insects in the Food Sector* (pp. 11-26). Springer.

Poma, G., Cuykx, M., Amato, E., Calaprice, C., Focant, J. F., & Covaci, A. (2017). Evaluation of hazardous chemicals in edible insects and insect-based food intended for human consumption. *Food and Chemical Toxicology, 100*, 70-79.

Reddivari, L., Hale, A. L., & Miller, J. C. (2007). Determination of phenolic content, composition and their contribution to antioxidant activity in specialty potato selections. *American Journal of Potato Research, 84*(4), 275-282.

Roos, N., & Van Huis, A. (2017). Consuming insects: are there health benefits? *Journal of Insects as Food and Feed, 3*(4), 225-229.

Rumpold, B. A., & Schlüter, O. K. (2013). Potential and challenges of insects as an innovative source for food and feed production. *Innovative Food Science & Emerging Technologies, 17*, 1-11.

Sang, S., Lapsley, K., Jeong, W.-S., Lachance, P. A., Ho, C.-T., & Rosen, R. T. (2002). Antioxidative phenolic compounds isolated from almond skins (*Prunus amygdalus* Batsch). *Journal of Agricultural and Food Chemistry, 50*(8), 2459-2463.

Santos, D. I., Saraiva, J. M. A., Vicente, A. A., & Moldão-Martins, M. (2019). Methods for determining bioavailability and bioaccessibility of bioactive compounds and nutrients. In *Innovative thermal and non-thermal processing, bioaccessibility and bioavailability of nutrients and bioactive compounds* (pp. 23-54). Elsevier.

Schittko, U., Burghardt, F., Fiedler, K., Wray, V., & Proksch, P. (1999). Sequestration and distribution of flavonoids in the common blue butterfly *Polyommatus icarus* reared on *Trifolium repens*. *Phytochemistry, 51*(5), 609-614.

Shahidi, F., & Zhong, Y. (2015). Measurement of antioxidant activity. *Journal of functional foods, 18*, 757-781.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture, 16*(3), 144-158.

Sosa, D. A. T., & Fogliano, V. (2017). Potential of insect-derived ingredients for food applications. *Insect Physiology and Ecology. Rijeka: InTech*, 215-231.

Suh, H.-J., Kim, S.-R., Lee, K.-S., Park, S., & Kang, S. C. (2010). Antioxidant activity of various solvent extracts from Allomyrina dichotoma (Arthropoda: Insecta) larvae. *Journal of Photochemistry and Photobiology B: Biology, 99*(2), 67-73.

Van Huis, A. (2013). Potential of insects as food and feed in assuring food security. *Annual review of entomology, 58*, 563-583.

Wiesen, B., Krug, E., Fiedler, K., Wray, V., & Proksch, P. (1994). Sequestration of host-plant-derived flavonoids by lycaenid butterfly *Polyommatus icarus*. *Journal of Chemical Ecology, 20*(10), 2523-2538.

Yi, L., Lakemond, C. M. M., Sagis, L. M. C., Eisner-Schadler, V., van Huis, A., & van Boekel, M. A. J. S. (2013). Extraction and characterisation of protein fractions from five insect species. *Food chemistry, 141*(4), 3341-3348. https://doi.org/10.1016/j.foodchem.2013.05.115

CHAPTER 4. ANTIBACTERIAL BIOACTIVITY OF ACHETA DOMESTICUS AND FEED EXTRACTS

4.1 Abstract

Recently, increasing bacterial resistance to antibiotics and the need to replace synthetic preservatives has led to research on unconventional sources of antibacterial compounds, among them, insect extracts. In this study, antibacterial activity of house cricket (*A. domesticus*) extracts from two different farms (organic *Acheta* and commercial *Acheta*) that used different feed (organic feed and commercial feed, respectively) against *E. coli* and *L. innocua* was evaluated using the microwell method. Although neither the *A. domesticus* or feed extracts had antibacterial activity towards *E. coli,* the organic *Acheta* extract showed inhibition ($p<0.05$) towards *L. innocua* between a period of 4 to 12 h, and the commercial *Acheta* extract between a period of 4 to 8 h, which could be a result from the presence of phenolic acids and antimicrobial peptides. The feed extracts showed no inhibition for *L. innocua*. Further studies in the antibacterial properties of insect extracts could lead to the discovery of new antibacterial compounds with diverse applications.

4.2 Introduction

Antimicrobial activity of plant phenolic compounds has been extensively studied against several microorganisms, due to the increasing concern regarding microbial resistance to conventional antibiotic and antimicrobial treatments, and interest in developing clean label food preservatives in the food industry (Cushnie & Lamb, 2005; Daglia, 2012; Farhadi et al., 2019). Among all phenolic compounds, flavonoids (mainly flavones, flavonols, flavan-3-ols, and chalcones) report the highest antimicrobial activity through diverse mechanisms, attributed to their amphipathic features given by hydrophobic substituents such as alkylamino chains and the presence of the heterocyclic ring (Farhadi et al., 2019).

Although specific information regarding antimicrobial bioactivity of phenolic extracts from insects has not yet been reported, remarkable bioactivity has been demonstrated for insect peptides, whole body insect extracts, and insect products. For example, bee (*Apis mellifera*) propolis presents antimicrobial activity, which has been attributed to its high flavonoid content, specifically the presence of galanin and pinocembrin (Cushnie & Lamb, 2005). Due to the high resistance of

insects to infectious diseases, research of their defense mechanisms has been of great interest; this high resistance is mainly attributed to antimicrobial peptides (AMPs) present in insects. These bioactive cationic peptides (less than 100 amino acid residues each) are classified structurally as defensins, cecropins, drosocins, attacins, diptericins, ponericins, metchnikowins and melittins (Wu et al., 2018), exerting their antibacterial action through membrane disruption and interference with bacterial metabolism (Wu et al., 2018). Furthermore, Lee et al. (2013) demonstrated the strong antibiotic action of coprisin, a defensin-like peptide extracted from the dung beetle (*Copris tripartitus*), against *Staphylococcus aureus.* The authors also demonstrated that coprisin targets bacterial membrane exerting its antimicrobial action.

Given the little information known on the possible antibacterial properties of insect extracts, the aim of this study was to evaluate the antibacterial activity of *A. domesticus* extracts and their corresponding feeds, against two bacteria strains, *Escherichia coli* ATCC 25922 and *Listeria innocua* ATCC 33090, which are of importance to the food industry due to their role in foodborne diseases.

4.3 Materials and Methods

4.3.1 Raw materials and chemicals

All materials and chemical reagents were purchased from Fisher Scientific (Waltham, MA, USA) and Sigma Aldrich (St. Louis, MO, USA) unless specified. The two *Acheta domesticus* samples (6 weeks old), organic *Acheta* and Commercial *Acheta,* were obtained from two rearing farms, Aspire Food group (Austin, TX) and Ovipost, Inc. (Labelle, FL) respectively. Both cricket samples were shipped frozen and kept in a -20 ºC freezer until needed. The two cricket feed samples, Organic feed, and Commercial feed were obtained from Aspire Food group (Austin, TX) and Mazuri® exotic animal nutrition, respectively. *Escherichia coli* ATCC 25922 and *Listeria innocua* ATCC 33090 were obtained from the American Type Culture Collection (Manassas, VA, USA).

4.3.2 Determination of Antibacterial Activity

The antimicrobial activity against *Escherichia coli* ATCC 25922 and *Listeria innocua* ATCC 33090 of *A. domesticus* and feed extracts was determined following the method described by Aguilar-Toalá et al. (2020) with modifications by Malm and Liceaga (2021).

Bacterial stock cultures

The freeze-dried bacteria were inoculated for 24 h in Brain Heart Infusion (BHI) and Mueller-Hinton Broth (MHB) for *L. innocua* and *E. coli*, respectively, following the ATCC guidelines. These inoculums were used to prepare glycerol freezer stocks with a final concentration of 20 % (w/v) that were stored at -80 ºC until needed. For the assay, a liquid stock was prepared by transferring 50 µL of *L. innocua* and *E. coli* freezer stock into 5 mL of BHI or MHB, respectively. The liquid stock of each bacteria was grown to stationary phase, implying an incubation time of 24 h for *L. innocua* and 14 h for *E. coli.* According to plate counts, the concentration of each liquid stock was 1.1 x 10^9 cfu/mL for *L. innocua* and 3.5 x 10^8 cfu/mL for *E. coli*. From these liquid stocks the experimental stocks were prepared by taking an aliquot of 40 µL into 10 mL of BHI or MHB for *L. innocua* and *E. coli,* respectively, having a final working concentration of 4.5 x 10^6 cfu/mL (*L. innocua*) and 1.3 x 10^6 cfu/mL (*E. coli*). Fresh liquid and experimental stocks were prepared before each assay and used immediately.

Sample preparation

Sterile samples of *A. domesticus* and feed extracts were prepared by dissolving 20 mg of freeze-dried extract in 1 mL of distilled water. The solutions were then individually filtered-sterilized using a 0.22 µm filter (Thermo Scientific, Rochester, NY, USA)

Antibacterial determination

The antibacterial activity of *A. domesticus* and feed extracts was determined using 96-well sterile microplates. For the sample wells, 55 µL of each *A.domesticus* (organic *Acheta* and commercial *Acheta*) or feed sample (organic feed and commercial feed) was inoculated with 165 µL of experimental stock of *L. innocua* or *E.coli* (~10^6 cfu/mL), resulting in a final sample

concentration of 5 mg/mL in each well with a total volume of 220 µL (n=5). Positive control was prepared by adding 220 µL of the corresponding experimental stock to each well (n=5). Negative controls were prepared by adding 55 µL of solvent (distilled water) or 55 µL of each *A. domesticus* or feed sample with 165 µL of BHI or MHB for *L. innocua* or *E. coli* assay, respectively. Following preparation, microplates were incubated for 24 h at 37 ºC and optical density measurements (OD_{620nm}) were conducted at 0, 4, 8, 12 and 24 h. Plate count was also done for the positive control at 2, 8, 12 and 24 h. Growth curves of samples and positive control were created by plotting optical density vs. time.

4.3.3 Statistics

Statistical analysis of observed differences among means was performed using one-way analysis of variance (ANOVA), followed by Tukey's pairwise comparison of means at a 5 % significance level with the statistical software Minitab 18® (State College, PA, USA).

4.4 Results and discussion

4.4.1 Antibacterial activity

Plate count of the positive control of *L. innocua* and *E. coli* was measured at 2, 8, 12 and 24 h to stablish a relationship between number of cells and the optical density (Figure 4). From these plots we were able to corroborate that optical density could be used to measure the antibacterial bioactivity of the extracts on both cultures.

The effect of *A. domesticus* and feed extracts on the growth curve of *L. innocua* ATCC 33090 and *E. coli* ATCC 25922 over a 24 h period is shown in Figure 5. Using the microwell method we observed that the organic *Acheta* sample and the commercial *Acheta* sample were able to partially inhibit the growth of *L. innocua* between a period of 4 to 12 h and 4 to 8 h, respectively ($p<0.05$), as the OD of the wells containing the extracts was lower than the control, leading to a modification of the typical grow curve of *L. innocua* (Figure 5). The modification of the growth curve could imply that the presence of the *A. domesticus* extracts prolonged the lag phase duration, which is related to growth inhibition (Belda-Galbis et al., 2015). In addition, *L. innocua* started the stationary phase (12 h) at what appeared to be a lower cell concentration in the wells that were exposed to the *A. domesticus* extracts, especially in the wells containing the organic *Acheta* sample

($p<0.05$), confirming the partial inhibitory capacity of the extracts. This initial effect on *L. innocua* growth could be attributed to several of factors among them, the presence of phenolic compounds that were previously characterized for the samples, and the presence of antimicrobial peptides (AMPs) (Otvos, 2000; Papuc et al., 2017).

Extensive work on plant polyphenols has showed that flavonoids, mainly flavones, flavonols and proanthocyanidins and, non-flavonoids like phenolic acids, have antibacterial properties due to their capacity to interact with the cell wall components of gram-positive and gram-negative bacteria. This interaction affects the permeability, fluidity, ion transport and respiration of the cell leading to its death (Papuc et al., 2017). Additionally, several studies reported the ability of phenolics to inhibit biofilm formation by preventing bacterial attachment (Borges et al., 2012), as well as the ability to interfere in protein synthesis through the repression or stimulation of bacterial genes (Ulrey et al., 2014).

As mentioned previously in Chapter 3 of this thesis, the phenolic composition of the *A. domesticus* extracts was obtained resulting in 4 major phenolic acids: 4-hydroxybenzoic acid, *p*-coumaric acid, ferulic acid and syringic acid. These compounds could play a relevant role in bacterial inhibition. Although specific studies on antibacterial properties of insect phenolics have yet to be reported, the inhibitory ability of these individual phenolic acids has been previously studied.

Phenolic acids are thought to act through combined mechanisms at the membrane and cytoplasm (Pernin et al., 2018). As weak organic acids ($pKa \sim 4.2$) and due to their lipophilic characteristics, phenolic acids are thought to penetrate the cell membrane through passive diffusion in their undissociated form, disturbing the membrane and once in the cytoplasm they acidify the pH leading to protein denaturation (Campos et al., 2009). In a recent study, ferulic (500- 2000 μg/mL) and gallic (100- 1250 μg/mL) acids were reported to have antibacterial activity against *E. coli*, *L. monocytogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* as these compounds were able to induce irreversible change in the membrane properties of the bacteria tested (Borges et al., 2013). In another study, Campos et al. (2009) reported that hydroxybenzoic acids (4-hydroxibenzoic, protocatechuic, gallic, vanillic, and syringic acids) and in greater way hydroxycinnamic acids (*p*-coumaric, caffeic and ferulic acids) increased the cell membrane permeability of gram-positive bacteria (*Oenococcus oeni* and *Lactobacillus hilgardii*), leading to cell death. In addition to the antibacterial activity of the individual phenolic acids, a synergistic

effect was also reported for a combination of hydroxycinnamic acids (*p*-coumaric, ferulic, cinnamic and caffeic acids) in which the anti-listeria activity of the combination of these compounds corresponded to the sum of the effects observed with the individual acid (Wen et al., 2003). This synergistic effect is very important as in almost all foods, including *A. domesticus,* individual phenolics are rarely found, in fact, the food contains a mixture of various compounds and this could be more beneficial.

Although phenolic compounds appear to have an impact on the overall antibacterial action of the *A. domesticus* extracts, the inhibitory activity can't be solely attributed to the action of the major phenolic acids as both feeds had a higher concentration of these compounds, yet there was no significant inhibition (no major modification of the typical grow curve of *L. innocua* was observed). Because the feeds had higher phenolic concentration, yet not major inhibitory activity, it is possible that other bioactive compounds such as antimicrobial peptides contributed to the antibacterial activity of the *A. domesticus* extracts.

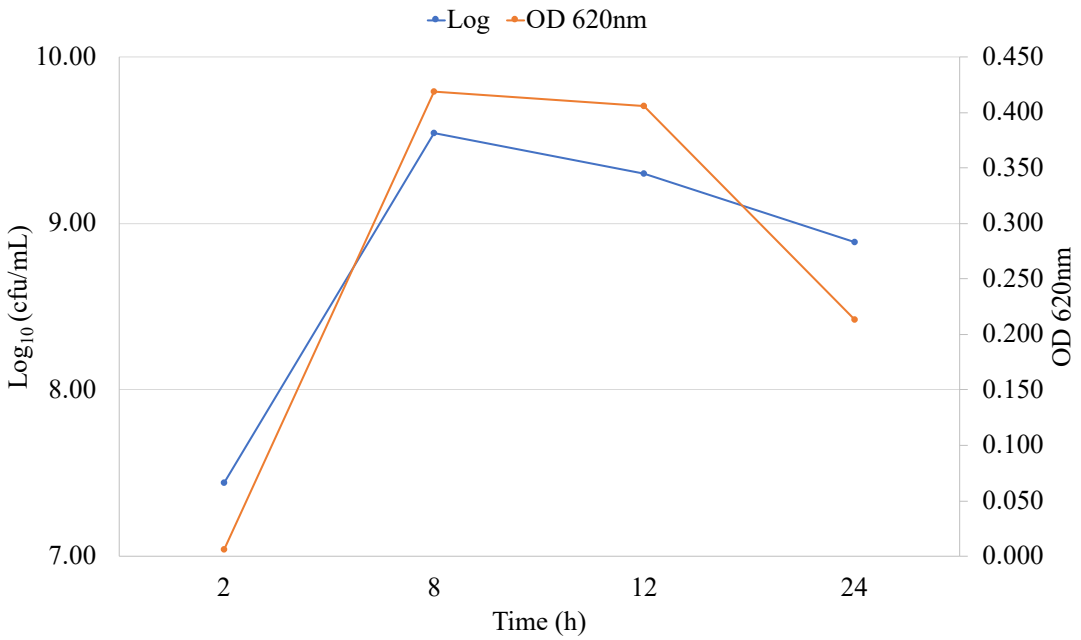
Insects have a complex defense mechanism. Upon detection of microorganisms, antimicrobial peptides are synthesized by the fat body and are then released to the hemolymph where they act to eliminate the threat (Bulet et al., 1999). Generally, antimicrobial peptides can be classified in three groups according to their structure and composition: 1) linear peptides forming α-helices and lack cysteine residues (Cecropins), 2) cyclic peptides stabilized by disulfide bridges due to the presence of cysteine residues (Defensins), and 3) peptides with an overrepresentation of proline and/or glycine residues (Bulet et al., 1999; Wu et al., 2018). These antimicrobial peptides are widely distributed in insects and are thought to be the main compounds involved in antibacterial activity of insect extracts (Kim et al., 2016). Extensive studies have been done in the action of antimicrobial peptides of Diptera characterizing seven peptides, among them defensins that have gram-positive antibacterial activity (Imler & Hoffmann, 2000). Other studies like the one published by Amer et al. (2019) have evaluated the antimicrobial capacity of whole insect extracts. In this study, ethyl acetate extracts of common green bottle fly (*Lucilia sericata*), blow fly (*Chrysomya albiceps*), and house fly (*Musca domestica*) showed antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* and *Staphylococcus epidermis* as well as for Gram-negative bacteria *E. coli* and *Klebsiella pneumoniae.* The authors attribute this antibacterial activity to the antimicrobial peptides present in the extracts.

Although *A. domesticus* extracts showed inhibition of *L. innocua* between a period of 4 to 12 h and 4 to 8 h (organic *Acheta* and commercial *Acheta*, respectively), the bacteria seem to adapt to the constituents of the sample recovering after 12 h of incubation, leading to the stabilization of the bacterial population in contrast with the control, where the bacteria appear to be entering into the death phase. This stabilization might be due to the high protein content of the samples, implying that the bacteria were able to utilize the different components of the sample as substrate.

In contrast, neither the *A. domesticus* extracts nor the feed extracts showed any inhibition of *E. coli* leading to conclude that the bacteria are not susceptible to any of the components of the samples and can use them as substrates (Figure 5). According to the data, it seems that the growth of the gram-positive bacteria *L. innocua* was affected by the *A. domesticus* extracts, while *E. coli* (gram-negative) is resistant. This selectivity could be related to the cell wall of the bacteria as extensive studies in plant polyphenols have demonstrated that gram-negative bacteria are less susceptible to polyphenols than gram-positive (Papuc et al., 2017). Additionally, insect antimicrobial peptides (mainly defensins) have shown antibacterial selectivity towards gram-positive bacteria due to the acidic character of the peptidoglycan layer that allows a better binding of positively charged peptides (Otvos, 2000). Once the antimicrobial peptides bind to the peptidoglycan layer, membrane permeability is disrupted, leading to its death (Otvos, 2000). Hou et al. (2007) have previously reported this selectivity towards gram-positive bacteria, where the extracts of the housefly (*Musca domestica*) exhibited greater antibacterial potential against *Staphylococcus aureus* and *Bacillus subtilis*, both gram-positive.

Further studies on *A. domesticus* extracts may lead to characterize the chemical compounds responsible for the antibacterial properties reported in the present study, allowing a possible application of this extract in food preservation. To the authors knowledge, this is the first time that antibacterial activity on *L. innocua* and *E. coli* is reported for *A. domesticus* extracts.

a)



b)

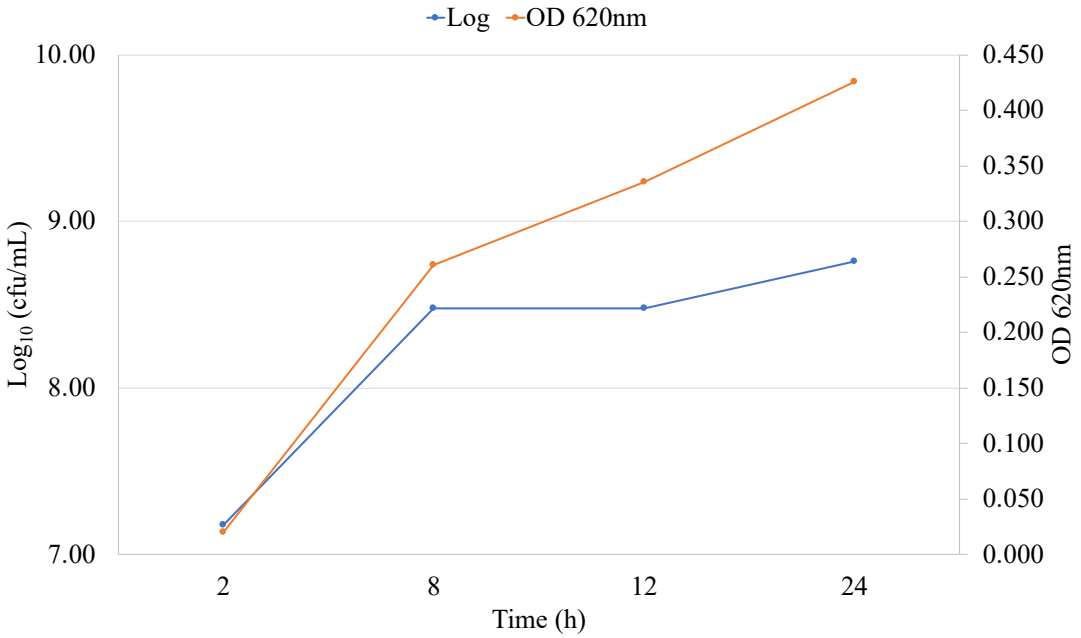


Figure 4. a) Growth curve of *L. innocua* measured in Log_{10} of the colony forming units (cfu) per mL (blue plot) and, OD 620nm (orange plot) over 24 hours of incubation (37ºC). b) Growth curve of *E. coli* measured in Log_{10} of the colony forming units (cfu) per mL (blue plot) and, OD 620nm (orange plot) over 24 hours of incubation (37°C).

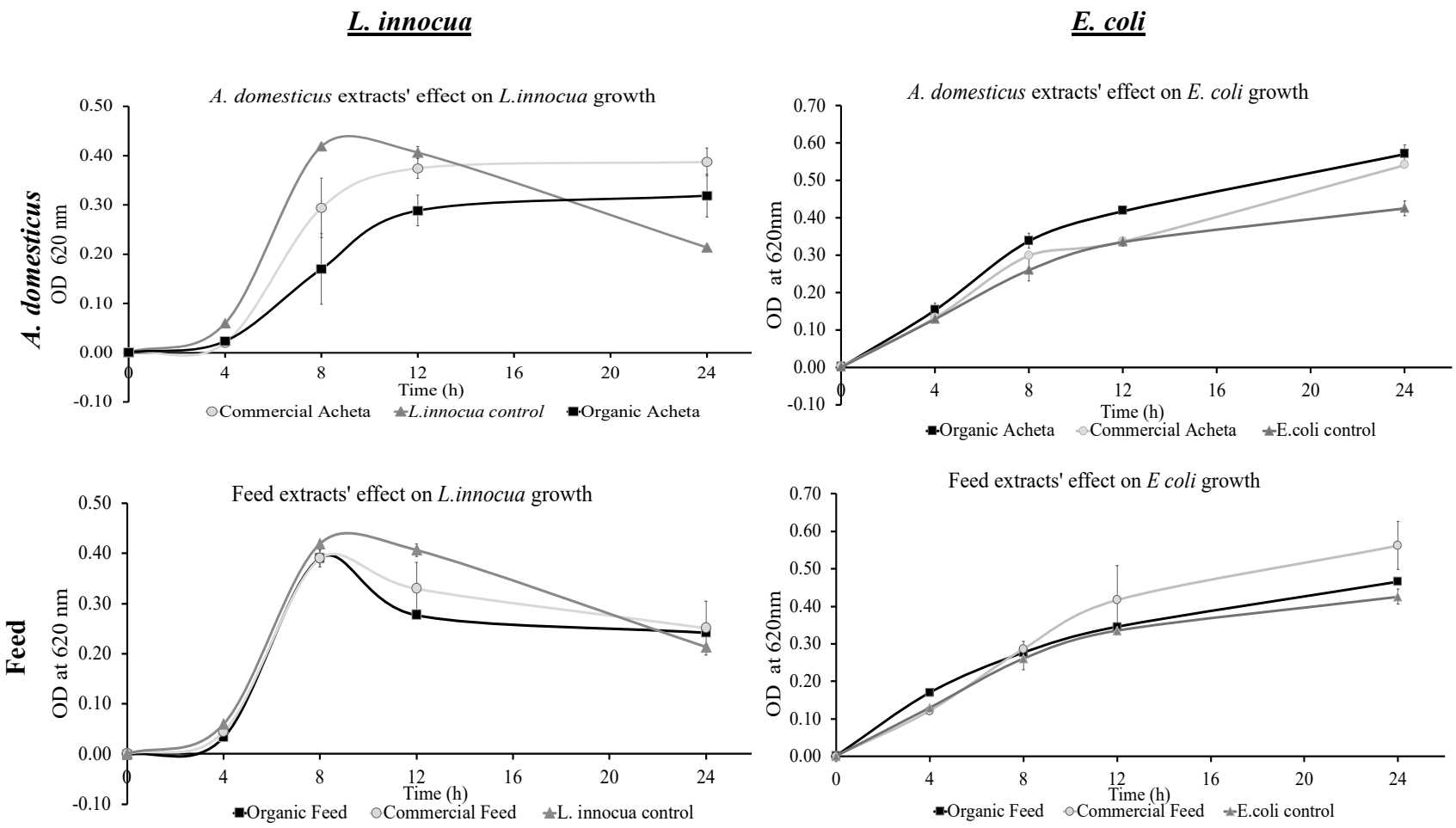


Figure 5. Effect of *A. domesticus* and feed extracts (5mg freeze-dried powder/mL) on the growth of Listeria innocua (left) and Escherichia coli (right) over 24 hours of incubation (37ºC).

4.5 Conclusion

The data presented in this study showed for the first time, the potential of the *A. domesticus* extracts to inhibit gram-positive bacteria such as *L. innocua*. The organic *Acheta* sample appears to have a greater inhibitory activity compared to the commercial *Acheta*. This observed antibacterial activity of both *A. domesticus* extracts is attributed to combination of the action of phenolic compounds and antimicrobial peptides, although further studies are needed to corroborate this hypothesis. More in-depth studies on *A. domesticus* extracts may lead to discover increased antibacterial action that could result in diverse applications.

4.6 References

Aguilar-Toalá, J. E., Deering, A. J., & Liceaga, A. M. (2020, 2020/12/01). New Insights into the Antimicrobial Properties of Hydrolysates and Peptide Fractions Derived from Chia Seed (Salvia hispanica L.). *Probiotics and Antimicrobial Proteins, 12*(4), 1571-1581. https://doi.org/10.1007/s12602-020-09653-8

Amer, M. S., Hammad, K. M., Shehata, A. Z., Hasballah, A. I., & Zidan, M. M. (2019). Antimicrobial and antiviral activity of Lucilia sericata, Chrysomya albiceps (Diptera: Calliphoridae) and Musca domestica (Diptera: Muscidae) whole body extract. *Egyptian Academic Journal of Biological Sciences. A, Entomology, 12*(2), 19-33.

Belda-Galbis, C. M., Jiménez-Carretón, A., Pina-Pérez, M. C., Martínez, A., & Rodrigo, D. (2015). Antimicrobial activity of açaí against Listeria innocua. *Food Control, 53*, 212-216.

Borges, A., Ferreira, C., Saavedra, M. J., & Simões, M. (2013). Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. *Microbial Drug Resistance, 19*(4), 256-265.

Borges, A., Saavedra, M. J., & Simões, M. (2012). The activity of ferulic and gallic acids in biofilm prevention and control of pathogenic bacteria. *Biofouling, 28*(7), 755-767.

Bulet, P., Hetru, C., Dimarcq, J.-L., & Hoffmann, D. (1999). Antimicrobial peptides in insects; structure and function. *Developmental & Comparative Immunology, 23*(4-5), 329-344.

Campos, F., Couto, J., Figueiredo, A., Tóth, I., Rangel, A. O., & Hogg, T. (2009). Cell membrane damage induced by phenolic acids on wine lactic acid bacteria. *International journal of food microbiology, 135*(2), 144-151.

Cushnie, T. T., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents, 26*(5), 343-356.

Daglia, M. (2012). Polyphenols as antimicrobial agents. *Current opinion in biotechnology, 23*(2), 174-181.

Farhadi, F., Khameneh, B., Iranshahi, M., & Iranshahy, M. (2019). Antibacterial activity of flavonoids and their structure–activity relationship: An update review. *Phytotherapy Research, 33*(1), 13-40.

Hou, L., Shi, Y., Zhai, P., & Le, G. (2007). Antibacterial activity and in vitro anti-tumor activity of the extract of the larvae of the housefly (Musca domestica). *Journal of ethnopharmacology, 111*(2), 227-231.

Imler, J.-L., & Hoffmann, J. A. (2000). Signaling mechanisms in the antimicrobial host defense of Drosophila. *Current opinion in microbiology, 3*(1), 16-22.

Kim, I.-W., Markkandan, K., Lee, J. H., Subramaniyam, S., Yoo, S., Park, J., & Hwang, J. S. (2016). Transcriptome profiling and in silico analysis of the antimicrobial peptides of the grasshopper Oxya chinensis sinuosa. *Journal of microbiology and biotechnology, 26*(11), 1863-1870.

Lee, E., Kim, J.-K., Shin, S., Jeong, K.-W., Shin, A., Lee, J., Lee, D. G., Hwang, J.-S., & Kim, Y. (2013). Insight into the antimicrobial activities of coprisin isolated from the dung beetle, Copris tripartitus, revealed by structure–activity relationships. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 1828*(2), 271-283.

Malm, M., & Liceaga, A. M. (2021). Physicochemical Properties of Chitosan from Two Commonly Reared Edible Cricket Species, and Its Application as a Hypolipidemic and Antimicrobial Agent. *Polysaccharides, 2*(2), 339-353.

Otvos, J., Laszlo. (2000). Antibacterial peptides isolated from insects. *Journal of peptide science: an official publication of the European Peptide Society, 6*(10), 497-511.

Papuc, C., Goran, G. V., Predescu, C. N., Nicorescu, V., & Stefan, G. (2017). Plant polyphenols as antioxidant and antibacterial agents for shelf-life extension of meat and meat products: Classification, structures, sources, and action mechanisms. *Comprehensive Reviews in Food Science and Food Safety, 16*(6), 1243-1268.

Pernin, A., Dubois-Brissonnet, F., Roux, S., Masson, M., Bosc, V., & Maillard, M. N. (2018). Phenolic compounds can delay the oxidation of polyunsaturated fatty acids and the growth of Listeria monocytogenes: structure-activity relationships. *Journal of the Science of Food and Agriculture, 98*(14), 5401-5408.

Ulrey, R. K., Barksdale, S. M., Zhou, W., & van Hoek, M. L. (2014). Cranberry proanthocyanidins have anti-biofilm properties against Pseudomonas aeruginosa. *BMC complementary and alternative medicine, 14*(1), 1-12.

Wen, A., Delaquis, P., Stanich, K., & Toivonen, P. (2003). Antilisterial activity of selected phenolic acids. *Food microbiology, 20*(3), 305-311.

Wu, Q., Patočka, J., & Kuča, K. (2018). Insect antimicrobial peptides, a mini review. *Toxins, 10*(11), 461.

CHAPTER 5. CONCLUSION AND FUTURE WORK

5.1 Conclusion

Phenolic extracts were successfully obtained from *A. domesticus* and feed using microwave-assisted extraction. These phenolic extracts were characterized by a targeted approach using UPLC/MS-MS. For both sets of extracts, *A. domesticus* and feed, the main phenolic compounds identified were 4-hydroxybenzoic acid, *p*-coumaric acid, ferulic acid and syringic acid. Given that the same main phenolic acids were identified in both extracts, the data suggest that *A. domesticus* may be able to absorb or sequester dietary phenolics. This is the first time that phenolic compounds are extracted, characterized, and quantified for farmed *A. domesticus*. Furthermore, *in vitro* antioxidant and antibacterial activity was reported for the microwave-assisted extracts of *A. domesticus* and feed. *A. domesticus* extracts exerted higher *in vitro* antioxidant activity than the plant-based feed extracts, possibly as a result of a synergistic interaction between phenolic compounds and proteins. In addition, the data presented in this study showed for the first time, the potential of the *A. domesticus* extracts to inhibit gram-positive bacteria such as *L. innocua.* This inhibitory activity could be the result of combined action of the phenolic compounds present in the extracts as well as antimicrobial peptides. A selective inhibition was observed for the gram-positive bacteria as the gram-negative *E. coli* was not affected by any of the extracts (*A. domesticus* or feed) suggesting that the peptidoglycan layer in *L. Innocua* is more susceptible to the extracts and their components. The phenolic characterization and the observed bioactivities of the *A. domesticus* extracts may lead to the discovery of potential health benefits of this insect when consumed.

5.2 Future work

Follow-up studies could confirm the absorption and sequestration of dietary phenolics by using starved crickets. This will allow a differentiation of the phenolic content absorbed by the insect and the phenolic compounds that were excreted and appear in the feces. In addition, the contribution of the sclerotization on the insect's phenolic profile could also give valuable information. Additionally, future studies could use an untargeted approach to the phenolic characterization using a LC-MS that could lead to identify more phenolic compounds in *A.*

domesticus as well as possible metabolites. This untargeted approach could also contribute to the understanding of the insect's metabolization of phenolic compounds.

As for the bioactivity evaluation of insect extracts, further characterization of the extracts could lead to pinpoint the bioactive compounds responsible for the observed *in vitro* antioxidant activity as well as the selective *L. innocua* inhibitory activity. Additionally, future studies could also focus on the evaluation of the bioaccessibility and bioavailability of these bioactive compounds. Simulated gastrointestinal digestion (static or dynamic) coupled with cell culture models (e.g Caco-2-cells) could be used to evaluate intestinal absorption ability of the insect extracts providing information regarding the bioaccessibility of bioactive compounds and, *in vivo* models could be used to determine the bioavailability.