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Insects as a source of phenolic compounds and potential health benefits

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REVIEW ARTICLE

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 metabolisation of the plant-derived phenolic present in their diet, but also from the ability of insects to synthesise 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 stabilise 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 characterisation 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.

Keywords: insect phenolics, antioxidant, anti-inflammatory, anticancer, antimicrobial

1. Introduction

Plant phenolic compounds are secondary metabolites characterised 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 recognised to have several bioactivities including antioxidant, anti-inflammatory, antimicrobial and anticancer (Carocho and 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, hypothesising that these were absorbed and/or metabolised from the diet and later incorporated in the body (Simmonds, 2003). However, phenolics in edible insects remain to be characterised 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. Insect phenolics

Sclerotisation as a source of phenolic compounds

Sclerotisation 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 synthesised 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 stabilisation 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 organised 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 synthesises a new one that will undergo sclerotisation. The general suggested mechanism (Figure 1) starts with tyrosine to synthesise 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 and Roepstorff, 1982) that can be further oxidised, 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 and 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.

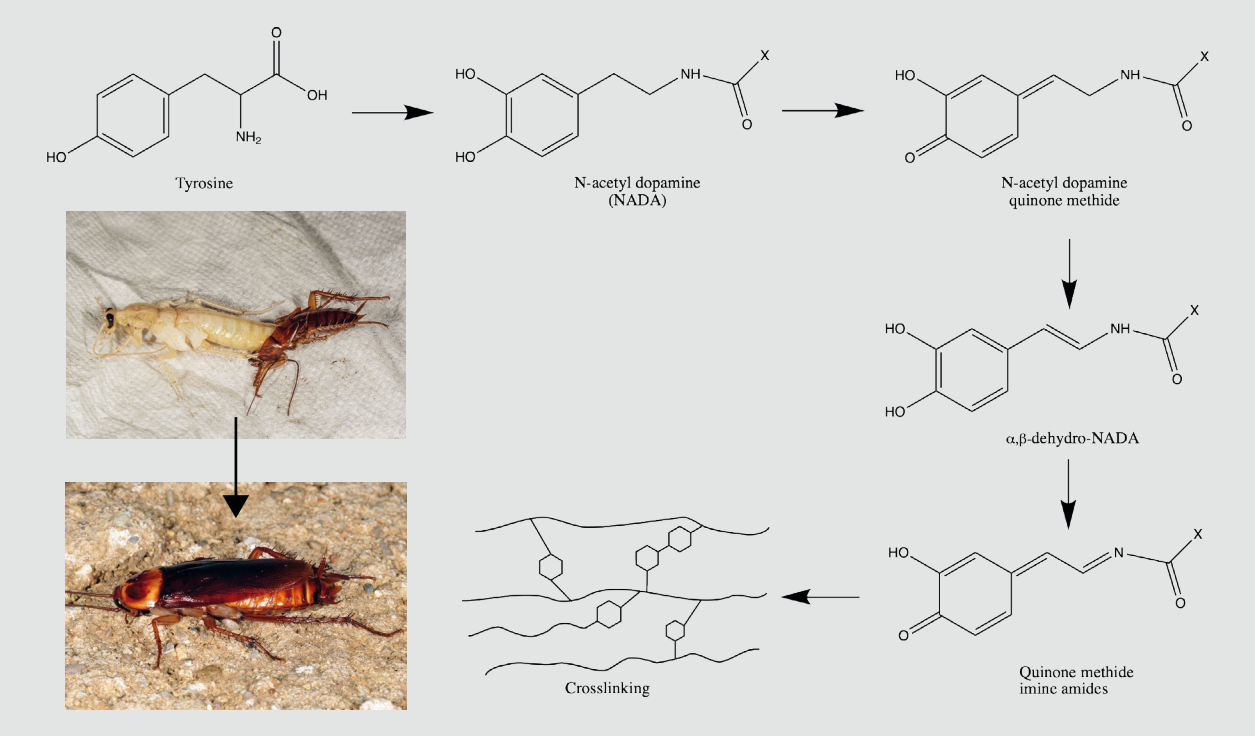

Figure 1. Simplified suggested mechanism of the sclerotisation process of insects (photo credit: John Obermeyer, Purdue University).

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 and 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).

The great majority of phenolic compounds found in insects are attributed to herbivore feeding behaviour. 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 and Lempa, 2002; Schittko *et al.*, 1999).

For example, interest in the characterisation 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 metabolise 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*), characterised 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 behaviour 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 characterisation 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

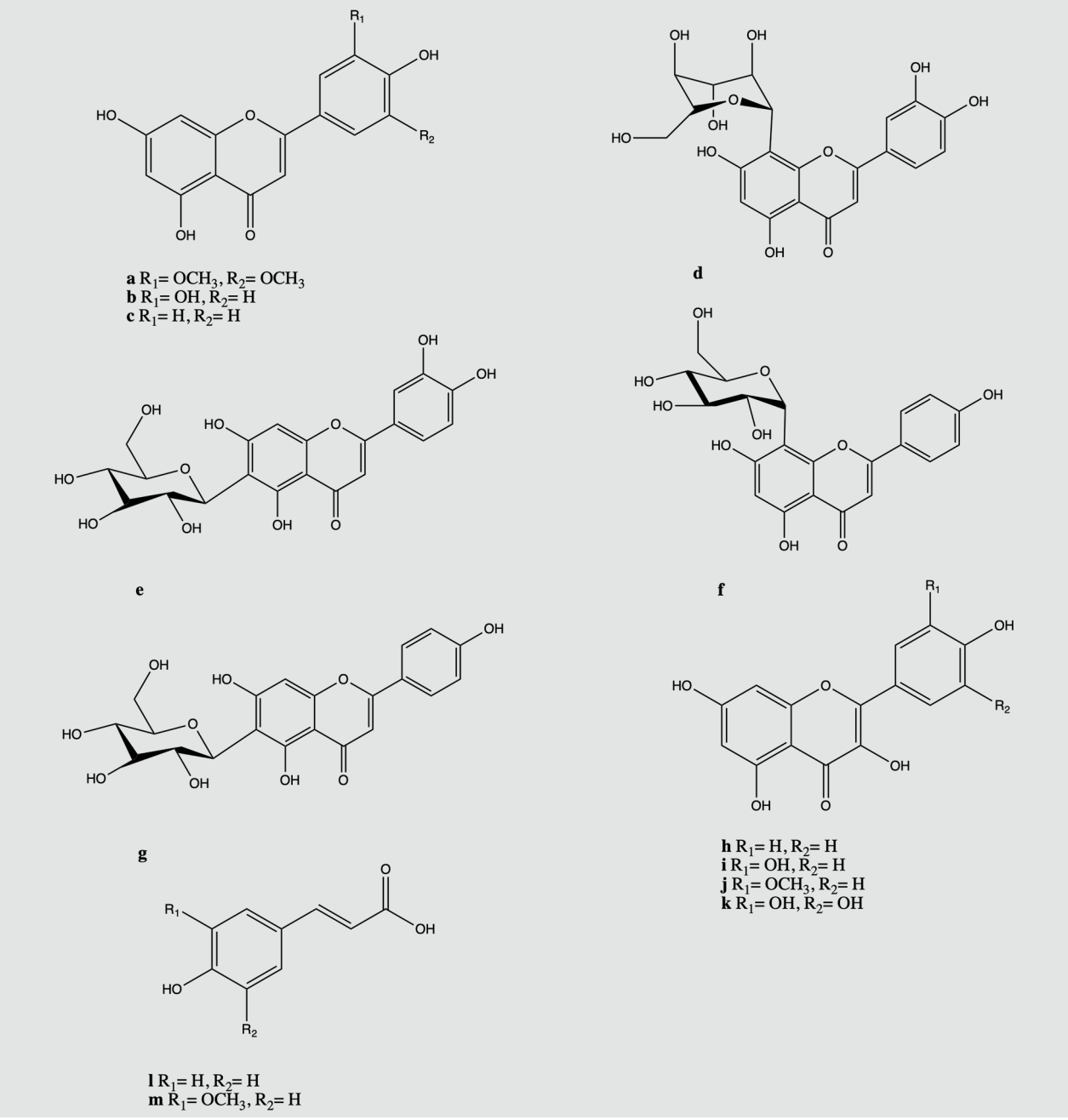

Figure 2. Phenolic compounds detected in insects: (A) tricin, (B) luteolin, (C) apigenin, (D) orientin, (E) iso-orientin, (F) vitexin, (G) isovitexin, (H) kaempferol, (I) quercetin, (J) isorhamnetin, (K) myricetin, (L) ferulic acid, (M) sinapic acid.

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 (*Dissosteira carolina*). The characterisation 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 and 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 metabolisation 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, 2008; Kurioka and Yamazaki, 2002; Tamura *et al.*, 2002). Ferreres *et al.* (2009b) analysed 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.*, 2008, 2009a).

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 analysed 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 mg GAE/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.

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.

3. 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 and 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*) (Zielińska *et al.*, 2017, 2018; Hall and Liceaga, 2020; Hall *et al.*, 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.

Antioxidant bioactivity

An antioxidant is a chemical compound that inhibits or slows down the oxidation of another compound (Shahidi and 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

Table 1. Phenolic compounds reported for different insect species.[1]

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.

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 and 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 ($O_2^{\cdot-}$), hydroxyl radical ($HO^{\cdot}$), alkoxyl radicals ($RO^{\cdot}$) and peroxyl radicals ($RO_2^{\cdot}$) (Amorati and 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 characterisation 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 optimising processing time and temperature and should be considered when processing insects (Şensoy *et al.*, 2006; Sikora *et al.*, 2008; Takeoka *et al.*, 2001).

Other potential bioactivities

Anti-inflammatory, anticancer and antimicrobial bio-activities 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 and Yeo, 2018). In one study, quercetin showed antitumor activity in prostate cancer cells involving the regulation of tumour 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 tumours in rats, and an up-regulation in antioxidant enzymes such as catalase and glutathione peroxidase (Nirmala and 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 and 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).

4. Conclusion

Incorporation of phenolic compounds in insects due to their capacity to sequester and metabolize dietary phenolics has been proven through the characterisation 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.

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Conflict of interest

The authors declare no conflict of interest.

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