



## ***In vitro* Antioxidant Activity of Edible Insects**

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### **Abstract**

Since consuming insects typically has a higher dietary potential, entomophagy presents a nutritional opportunity. Insects have a long history of usage as nutritious and sustainable food, but little is known about their dietary antioxidants. In this work, antioxidant activity was tested *in vitro* using five edible insects: Meal worms, Black ants, Crickets, Grasshoppers, and Silk worms. In this study, the gastrointestinal digestion and absorption of edible insects (raw sample) *in vitro* were used to examine the insects' antioxidant characteristics. The Radical Scavenging Activity for Meal worms (63.64  $\mu\text{g/ml}$ ), Black ants (79.06  $\mu\text{g/ml}$ ), Crickets (73.50  $\mu\text{g/ml}$ ), Grasshoppers (60.85  $\mu\text{g/ml}$ ), and Silk worms (68.17  $\mu\text{g/ml}$ ). DPPH radical (DPPH•) scavenging activity for Meal worms (66.65  $\mu\text{g/ml}$ ), Black ants (86.33  $\mu\text{g/ml}$ ), Crickets (77.11  $\mu\text{g/ml}$ ), Grasshoppers (63.07  $\mu\text{g/ml}$ ) and Silk worms (71.17  $\mu\text{g/ml}$ ). TPI, the Total Polyphenol Index of edible insects' values expressed as Meal worms (253 mgGAE/100g), Black ants (178 mgGAE/100g), Crickets (193 mgGAE/100g), Grasshoppers (272 mgGAE/100g) and Silk worms (214 mgGAE/100g).  $\text{Fe}^{2+}$  Chelating Activity was reported for Meal worms (74.61  $\mu\text{g/ml}$ ), Black ants (88.39  $\mu\text{g/ml}$ ), Crickets (82.67  $\mu\text{g/ml}$ ), Grasshoppers (67.16  $\mu\text{g/ml}$ ), and Silk worms (77.48  $\mu\text{g/ml}$ ), whereas in Ferric Reducing Antioxidant Power (FRAP) reported for Meal worms (72.22  $\mu\text{g/ml}$ ), Black ants (88.40  $\mu\text{g/ml}$ ), Crickets (84.29  $\mu\text{g/ml}$ ), Grasshoppers (64.75  $\mu\text{g/ml}$ ) and Silk worms (76.94  $\mu\text{g/ml}$ ). The study emphasises the tremendous potential of bioactive chemicals and antioxidant components found in methanolic extracts of edible insects, which are safe and affordable food supplements. Future research should place greater attention on insects' ability to reduce oxidative stress through their antioxidant capacity.

**Keywords:** Antioxidant activity,  $\text{Fe}^{2+}$  Chelating Activity Edible insects, entomophagy, Total Polyphenols Index, DPPH, FRAP, Radical Scavenging Activity.

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### **1. Introduction**

It is anticipated that as demand for more sustainable protein sources rises in the future years, edible insect consumption will increase. Compared to other, more conventional sources of protein like cattle or pigs, insects need less space, food, and water to survive. Their carbon impact is far smaller, and they reproduce considerably more frequently (Van Huis *et al.*, 2013). The amount of  $\text{NH}_3$  emissions from insects, which contribute to soil nitrification and acidity, is much smaller than that of animals. Insects have a better feed conversion efficiency because they are properly recognized (Oonincx *et al.*, 2010). Apart from helping the environment, insects are a far more nutrient-dense food source than conventional protein

sources. They have substantial protein content, varying from 35% to 60% in Isoptera and Orthoptera and the composition of their amino acid satisfies the essential amino acid necessities of humans (Liceaga *et al.*, 2021). Better sources of variable minerals like iron and magnesium are contained in a lot of polyand monounsaturated fats and are a worthy source of numerous minerals, like iron, magnesium, phosphorus, and folic acid (Rumpold and Schluter, 2013). Because of their alleged antioxidant activity, capacity to suppress enzymes linked to hypertension, and type-2 diabetes, and anti-inflammatory qualities, edible insects have recently attracted interest for their possible medicinal benefits (Liceaga *et al.*, 2021).

It has been discovered that bioactive peptides take part in interfaces of peptide-enzyme which disrupt the structure of enzymes and impair their ability to carry out metabolic functions. Moreover, they have been revealed to have a function in cholesterol metabolism by eliminating compounds that cause hyperlipidaemia and can control the gene countenance for non-standard signaling pathways (Howard and Udenigwe, 2013). Food proteins include bioactive peptides that may have benefits for lowering blood pressure, swelling, diabetes of type 2, infections of microbes, immunological diseases, and oxidation (Urbizo-Reyes *et al.*, 2019). It is believed that oxidation, a process that takes place inside human cells and is characterised by free radical production, aids in the transmission of intracellular information (Durackova, 2010). While oxidation can occasionally be beneficial, when it spirals out of control, it can destroy important components at the cellular level and result in illness (Sanchez & Vazquez, 2017). Antioxidants are well-known substances that fight these harmful effects. Alpha-lipoic acid is one of the antioxidants that the human body can manufacture on its own, but it is advised that we consume more antioxidants in our diet to slow the rate of oxidative damage to our cells (Packer *et al.*, 1995).

Although the body produces free radicals regularly as a result of normal metabolic processes, while there is a disparity between the formation of reactive oxygen species (ROS) and the capacity of cells to neutralise, oxidative stress develops. Oxidative stress contributes to the onset of what is called "development illnesses" like Parkinson's and Alzheimer's as well as the degenerative process of aging. These diseases include cancer, stroke, myocardial infarction, and inflammation (Stadtman, 2006; Ali *et al.*, 2008). Antioxidant-rich dietary consumption is crucial in the prevention of many illnesses. Many studies have demonstrated the protective benefits of antioxidant and anti-inflammatory peptides contrary to ROS, which may help to significantly lower the amount of oxidative stress (Zielinska *et al.*, 2017; Karas *et al.*, 2015; Carrasco-Castilla *et al.*, 2012).

So far, there is no proof in the literature that the antioxidant qualities of insects and other invertebrates that are edible for humans have been screened. To demonstrate an antioxidant effect *in vitro*, we analyse oxidative stress produced by commercially available edible insects of diverse species and feeding behaviours.

## **2. Materials and Methods**

### **2.1 Insects Samples**

These five normal bug-type worms, Meal's Black ants, Crickets, Grasshoppers, and Silk worms were investigated. These bug specimens were purchased from nearby business merchants.

## 2.2 Edible Insect Preparation

The five different insect types were subjected to fasting for a day before emptying their gastrointestinal tracts of remaining foodstuff. There were no heat treatments applied to any of the bug species. Lastly, the insects (approximately 500 g) were lyophilized, coated with ice, and kept at  $-20^{\circ}\text{C}$  for additional research.

## 2.3 Radical Scavenging Activity (TEAC)

To assess the 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfuric acid) radical cation decoloration test's capacity to scavenge free radicals, according to Re et al.'s 1999 publication. With Perkin Elmer Lambda Bio 20 spectrophotometer (Boston, MA, USA), at 734nm, ABTS<sup>+</sup>•'s bleaching rate in the existence of the sample was observed. For the analysis of the extracts of aqueous and polar, the ABTS<sup>+</sup>• stock solution was diluted in either water or ethanol up to an Abs of  $0.70 \pm 0.02$ , respectively. For hydrophilic and lipophilic extracts, 2.97 and 2.88 L of ABTS<sup>+</sup>• solution were employed, respectively. To initiate hydrophilic and lipophilic extracts 30 and 120  $\mu\text{L}$ , respectively, were added to aqueous and ethanolic ABTS solutions. At  $30^{\circ}\text{C}$  as the ABTS<sup>+</sup>• bleaching was observed, the decolorization after 5 minutes was used as a gauge of antioxidant activity. The amount of Trolox Equals Antioxidant Capacity (mmol of Trolox eq. per 100 g of sample) used to measure the effectiveness of radical scavenging was calculated by dividing the correlation coefficient between the dose-response curves of the reference compound and the sample. The radical scavenging action for both the water-soluble (TEAC<sub>aq</sub>) and the liposoluble extracts was calculated using Trolox as the reference (TEAC<sub>lipo</sub>).

## 2.4 DPPH Radical Scavenging Activity Assay

The investigation of the DPPH radical (DPPH•)peptide fractions' scavenging activity by Brand-Williams, Cuvelier, and Berset (1995) concerning the concentrations of the antioxidant solution was somewhat adjusted by Karas *et al.* (2015). The material was added to 0.96 mL of a 6 M solution of DPPH in 75% methanol in a volume of 0.04 mL. At 515 nm, the absorbance was measured 3 minutes after the process began. As a blank, 75% methanol was utilised. Equation (1) was used to compute the scavenging effect.

## 2.5 Total Polyphenols Index (TPI)

The Giacintucciet *al.*, 2016 technique was adjusted to determine the TPI. Before adding 500  $\mu\text{L}$  of the Folin-Ciocalteu reagent, the sample extract (0.1 mL) was diluted in deionized water to a concentration of 5 mL. After three minutes, 1.5 mL of a 25%  $\text{Na}_2\text{CO}_3$  solution was added, then 10 mL of deionized water. Solutions were held at room temperature and in the dark for 60 minutes to calculate the total quantity of polyphenols present using a Perkin Elmer Lambda Bio 20 spectrophotometer. For calibration, standard solutions of gallic acid (Fluka, Buchs, CH) were utilised. Milligrams of gallic acid equivalents (GAE) per 100 g of material were used to express the results.

## 2.6 Determination of $\text{Fe}^{2+}$ Chelating Activity

With a few minor adjustments, the Hu *et al.*, 2012 procedure was used for the  $\text{Fe}^{2+}$  chelation experiment (Karas *et al.*, 2015). In a nutshell, 1 mL of the sample was combined

with a 2 mM FeCl<sub>2</sub> solution and a 5 mM ferrozine solution. The mixture was vigorously stirred. The absorbance at 562 nm was measured after 10 minutes of incubation in the room.

### 2.7 Ferric Reducing Antioxidant Power (FRAP)

The approach of Benzie and Strain (1996) was slightly altered to ascertain the materials' reducing activity. To create the FRAP reagent, 10 mM of TPTZ (2,4,6-tripyridyl-s-triazine) solubilized in 40 mM of HCl and 20 mM of FeCl<sub>3</sub> were combined. As a result, 2,900 L of reagent was produced. The change in absorbance was observed at 593 nm for six minutes. Using a calibration plot based on FeSO<sub>4</sub>, the data were presented as moles of Fe<sup>2+</sup> per 100 grams of defatted weight. The wavelength of 7H<sub>2</sub>O was 562 nm.

### 2.8 Calculation for %IC<sub>50</sub> (Inhibition concentration 50 percent)

$$(\%IC_{50}) = [1 - A \text{ sample}/A \text{ control}] \times 100 \quad \dots\dots\dots (1)$$

where the absorbance of the sample and control are specified as A sample and A control.

The IC<sub>50</sub> value was determined from the findings (inhibitory concentration). The peptide dose at which the inhibition percentage fell to 50% was extrapolated to estimate the IC<sub>50</sub> after numerous dilutions of each sample were tested for their ability to scavenge free radicals. The graph depicting scavenging activity for the four varied peptide contents was utilised to get the IC<sub>50</sub> value.

## 3. Results and Discussion

Even though some people still find the practice of "entomophagy" to be unpleasant, it is more common in European countries for humans to ingest invertebrates intact. Although it is widely acknowledged that insects are a trustworthy supply of superior proteins, minerals, vitamins, and fatty acids with no negative ecological effect, little is known about their function as a source of bioactive compounds. We have demonstrated that market-existing edible insects can be a source of antioxidant chemicals, albeit to varying degrees of effectiveness, depending on the taxonomy and feeding habits of the insects. Antioxidant-rich meals, such as fruit, and vegetables, help people avoid oxidative stress-related diseases like cancer, diabetes, and cardiovascular disease (Magrone *et al.*, 2013). The bioavailability of antioxidant-rich foods and the presence of chronic oxidative stress has a significant impact on their *in vitro* effectiveness (Lettieri-Barbato *et al.*, 2013; Serafini *et al.*, 2011; Serafini *et al.*, 2003). a significant number of antioxidants. However, for a preliminary evaluation of the antioxidant potentiality of novel meals, a high antioxidant content in the food matrix is essential. They have discussed the results of some studies involving edible insects and antioxidant capacity, even though it might be difficult to gauge an antioxidant's effectiveness in a test tube whether it is high or low.

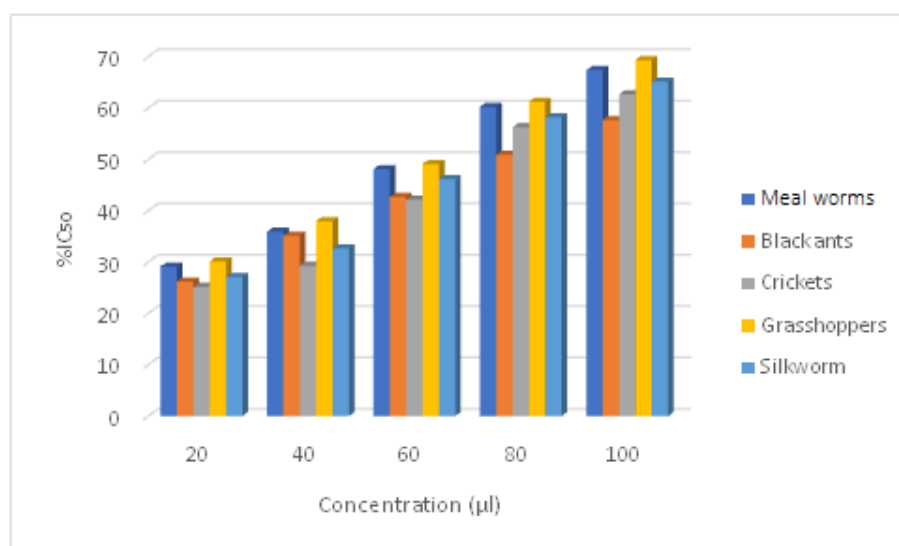
### 3.1 Radical Scavenging Activity (TEAC)

The five edible insects they are Meal worms, Black ants, Crickets, Grasshoppers, and Silk worms were examined for *in-vitro* antioxidant activity. The IC<sub>50</sub> value for the edible insects is Meal worms(63.64 µg/ml), Black ants(79.06 µg/ml), Crickets(73.50 µg/ml),

Grasshoppers(60.85  $\mu\text{g/ml}$ ), and Silk worms(68.17  $\mu\text{g/ml}$ ). The highest inhibition concentration shows in Grasshoppers and next to it are Meal worms. The Grasshoppers display the highest values of antioxidant capacity, measured as TEAC<sub>aq</sub> (mmol TE/100 g). The TEAC (Trolox Equivalent Antioxidant Capacity) of water-soluble insect extracts for human consumption. The values are the mean  $\pm$  SD of three replicates and are given as TEAC<sub>aq</sub> (mmolTE/100 g of defatted sample or 100 mL). Trolox Equivalents, or TE.

**Table 1: Radical Scavenging Activity (TEAC)**

Concentration	% IC <sub>50</sub>				
	Meal worms	Black ants	Crickets	Grasshoppers	Silk worms
20	29.14	26.22	25.14	30.14	27.06
40	35.93	35.12	29.25	37.98	32.66
60	48.11	42.67	42.18	49.11	46.17
80	60.24	50.89	56.27	61.21	58.22
100	67.38	57.63	62.68	69.37	65.11



**Figure 1: Radical Scavenging Activity (TEAC)**

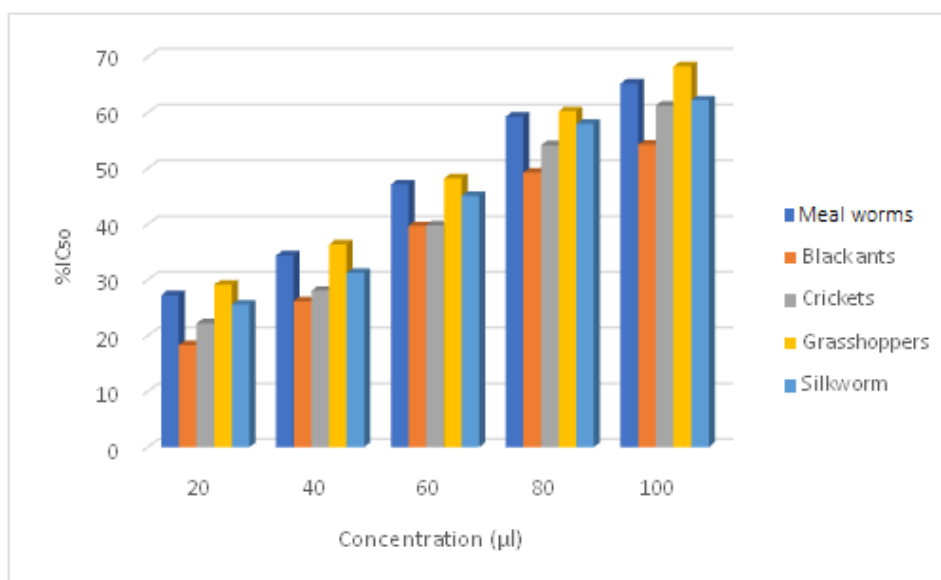
The findings of the current investigation demonstrate that insect heat treatment improved the hydrolysates' antiradical activity and, and that baking was associated with superior outcomes. This is because the insect peptide's sequence is altered during heat treatment (You *et al.*, 2012). The issue of peptides with antioxidant properties was affected by the heat treatment method, it should be inferred based on the results. According to the findings provided by Karas *et al.*, in 2015, heat treatment has a beneficial effect on the antioxidant activity of chickpea seeds. Moreover, these outcomes are consistent with those seen for beef meat (Fu *et al.*, 2017). Lipo-soluble extracts of edible insects have a high TEAC (Trolox Equivalent Antioxidant Capacity).

### 3.2 DPPH Radical Scavenging Activity Assay

The IC<sub>50</sub> value for the edible insects is Meal worms (66.65 µg/ml), Black ants (86.33 µg/ml), Crickets (77.11 µg/ml), Grasshoppers (63.07 µg/ml), and Silk worms (71.17 µg/ml). The highest inhibition concentration shows in Grasshoppers and next to it are Meal worms.

**Table 2: DPPH Radical Scavenging Activity Assay**

Concentration	% IC <sub>50</sub>				
	Meal worms	Black ants	Crickets	Grasshoppers	Silk worms
20	27.24	18.27	22.19	29.11	25.57
40	34.37	26.15	28.06	36.33	31.22
60	47.11	39.64	39.77	48.18	45.06
80	59.26	49.21	54.15	60.24	57.98
100	65.22	54.22	61.24	68.29	62.21



**Figure 2: DPPH Radical Scavenging Activity Assay**

The release of peptides with antioxidant properties was affected by the heat treatment method, it should be inferred based on the results. According to the findings provided by Kara *et al.*, in 2015, heat treatment has a beneficial effect on the antioxidant activity of chickpea seeds. Moreover, these outcomes are consistent with those seen for beef meat (Fu *et al.*, 2017). The isolated peptides from cooked meat had DPPH• scavenging activity that was even 20% greater than that of peptides from raw meat.

### 3.3 Total Polyphenols Index (TPI)

Meal worms (253 mgGAE/100g), Black ants (178 mgGAE/100g), Crickets (193 mgGAE/100g), Grasshoppers (272 mgGAE/100g), and Silk worms (214 mgGAE/100g) are the values for the Total Polyphenol Index (TPI) of edible insects. These results are expressed as the mean SD in triplicate. GAE stands for gallic acid equivalent. Except for grasshoppers,

our samples' phenolic content, as determined by TPI demonstrates that their antioxidant activity is not only due to this class of chemicals. Since that other authors have already shown that proteins play a role in their antioxidant action, they likely do (Zielinska *et al.*, 2017; Hall *et al.*, 2018; Nongonierma and Fitzgeralds, 2017).

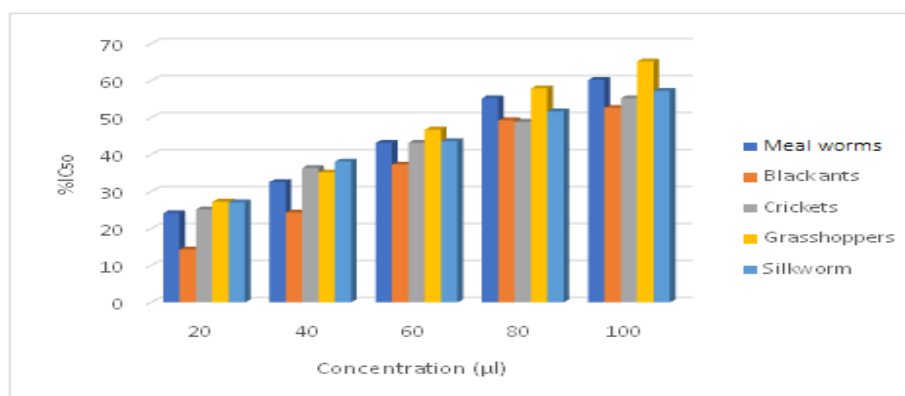
Yet, our results are substantially better than those of orange juice if we consider that the FRAP test does not account for the antioxidant contribution from protein groups. Our research indicates that edible insects include a particular pattern of redox elements, including phenolics, proteins, and unknown substances, that can mitigate oxidative stress caused by water and a lipophilic environment. Ascorbic acid and water-soluble phenolics are also the primary antioxidants in orange juice, while tocopherols and amphiphilic phenolics are the primary antioxidants in olive oil.

### 3.4 Determination of Fe<sup>2+</sup> Chelating Activity

Among all the hydrolysates examined, the various edible insect protein preparation hydrolysate demonstrated the highest ability to chelate Fe<sup>2+</sup> ions. Meal worms (IC<sub>50</sub> = 74.61 g/ml), Black ants (88.39 g/ml), Crickets (82.67 g/ml), Grasshoppers (67.16 g/ml), and Silk worms (IC<sub>50</sub> = 77.48 g/ml) are edible insects. Grasshoppers exhibit the highest inhibitory concentration, followed by Meal worms. In general, the insects' heat treatment improved the hydrolysates' and peptide fractions' capacity to chelate Fe<sup>2+</sup>.

**Table 3: Determination of Fe<sup>2+</sup> Chelating Activity**

Concentration	% IC <sub>50</sub>				
	Meal worms	Black ants	Crickets	Grasshoppers	Silk worms
20	24.17	14.29	25.19	27.24	27.06
40	32.58	24.31	36.38	35.16	38.11
60	43.16	37.28	43.16	46.76	43.61
80	55.24	49.25	48.88	57.93	51.67
100	60.23	52.67	55.24	65.24	57.24



**Figure 3: Determination of Fe<sup>2+</sup> Chelating Activity**

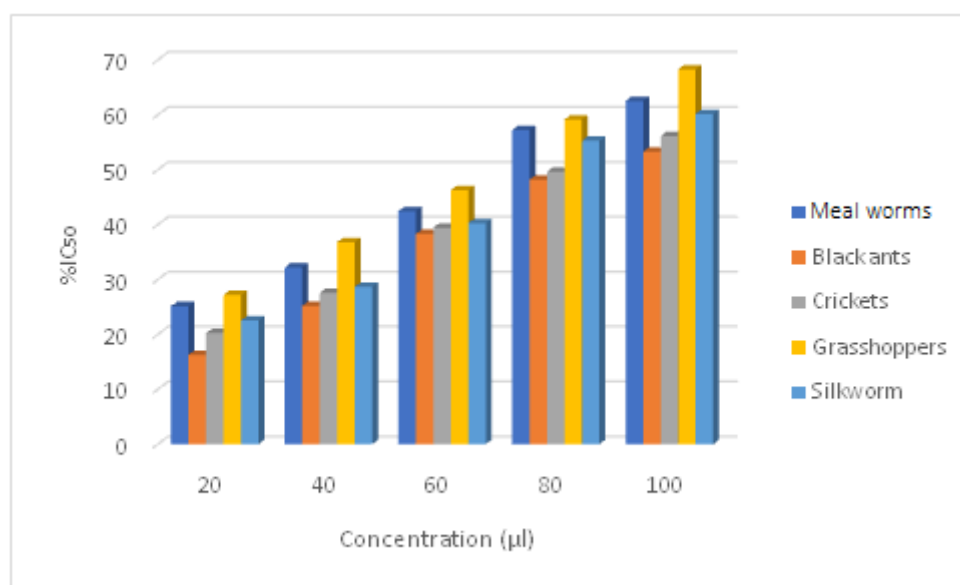
The use of heat processing has a significant impact on the issue of physiologically active peptides during in vitro gastrointestinal protein absorption (Karas *et al.*, 2015). The distinctive peptide structure and amino acid side chain groups of insect peptides, which were essential in halting free radical chain reactions and chelating transition-metal ions, may be responsible for their strong chelating activity (Zhu *et al.*, 2008). Another discovery was that baked insects had the strongest propensity to chelate  $\text{Fe}^{2+}$ . These results concur with those reported by Karas *et al.*, in 2015. With an  $\text{IC}_{50}$  value of 2.03 mg/mL, Wu *et al.* (2011) obtained a different result for Silk worms protein hydrolysate.

### 3.5 Ferric Reducing Antioxidant Power (FRAP)

In general, samples following the absorption process and protein preparations among the hydrolysates showed the lowest values. Also, it appears that among the peptide components, greater reduction power is the predominant effect of heating insects. The  $\text{IC}_{50}$  value for the edible insects is Meal worms (72.22  $\mu\text{g}/\text{ml}$ ), Black ants (88.40  $\mu\text{g}/\text{ml}$ ), Crickets (84.29  $\mu\text{g}/\text{ml}$ ), Grasshoppers (64.75  $\mu\text{g}/\text{ml}$ ), and Silk worms (76.94  $\mu\text{g}/\text{ml}$ ). The highest inhibition concentration shows in Grasshoppers and next to it are Meal worms.

**Table 4: Ferric Reducing Antioxidant Power (FRAP)**

Concentration	% $\text{IC}_{50}$				
	Meal worms	Black ants	Crickets	Grasshoppers	Silk worms
20	25.13	16.27	20.21	27.15	22.53
40	32.17	25.20	27.53	36.79	28.64
60	42.45	38.31	39.42	46.24	40.27
80	57.21	48.16	49.63	59.13	55.24
100	62.53	53.24	56.14	68.26	60.16



**Figure 4: Ferric Reducing Antioxidant Power (FRAP)**



The results highlight the importance of edible insects and invertebrates as maintainable original nutriment from a dietary, useful, and environmental standpoint. They show that they are an ideal source of bioactive compounds as well as first-class protein, minerals, vitamins, and fatty acids, along with a little ecological influence (Van Huis *et al.*, 2013). In light of the growing interest in entomophagy among the scientific community, the media, and consumers, the results of our study are significant from the standpoint of public health as well. They will serve as the foundation for creating entomophagy-promotional programs that educate the public about the need to restrict the use of edible insect-based food that has a high ecological effect while upholding or uniformly boosting nutritious and practical assistance. Researchers may in the future create a customised dietary regimen for the growth of insects following the "one health" concept in instruction to rise antioxidant content and improve nourishing intake for a sustainable and beneficial animal or human diet.

#### 4. Conclusion

This study, in our opinion, is the first to use fermentation to create bioactive molecules that block antioxidants from food sources. These findings suggest that the heat-treatment technical method has a considerable impact on the protein's accessibility for enzymatic digestion, which raises the content of antioxidant peptides. It is important to underline the potential health benefits of eating edible insects and consuming food that has been altered to include insect proteins or lipids. Entomophagy may very well be demonstrated to be a valuable technique in preventing diseases, even though consuming edible insects may potentially prevent diseases associated with oxidative stress. Even if eating edible insects may potentially prevent diseases linked to oxidative stress, entomophagy may very well be proven to be a useful strategy in preventing diseases that threaten civilization. To determine whether eating insects and other invertebrates may help to reduce oxidative stress in humans as well as to identify the bioactive components present in these species, more research is needed.

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