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 µg/ml), Black ants (79.06 µg/ml), Crickets (73.50 µg/ml), Grasshoppers (60.85 µg/ml), and Silk worms (68.17 µg/ml). DPPH radical (DPPH•) scavenging activity for 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). 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). Fe^{2+} Chelating Activity was reported for Meal worms (74.61 µg/ml), Black ants (88.39 µg/ml), Crickets (82.67 µg/ml), Grasshoppers (67.16 µg/ml), and Silk worms (77.48 µg/ml), whereas in Ferric Reducing Antioxidant Power (FRAP) reported for Meal worms (72.22 µg/ml), Black ants (88.40 µg/ml), Crickets (84.29 µg/ml), Grasshoppers (64.75 µg/ml) and Silk worms (76.94 µg/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, Fe^{2+} Chelating Activity Edible insects, entomophagy, Total Polyphenols Index, DPPH, FRAP,Radical Scavenging Activity.

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 NH_3 emissions frominsects, 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(Rumpoldand 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°C for additional research.

2.3 Radical Scavenging Activity (TEAC)

To assess the 2,2′-azino-nis (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+•'s bleaching rate in the existence of the sample was observed. For the analysis of the extracts of aqueous and polar, the ABTS+• stock solution was diluted in either water or ethanol up to an Abs of 0.70 ± 0.02, respectively. For hydrophilic and lipophilic extracts, 2.97 and 2.88 L of ABTS+• solution were employed, respectively. To initiate hydrophilic and lipophilic extracts 30 and 120 µL, respectively, were added to aqueous and ethanolic ABTS solutions. At 30^{o}C as the ABTS+• bleaching was observed, the decolourization 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 (TEACaq) and the liposoluble extracts was calculated using Trolox as the reference (TEAClipo).

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 Giacintucci*et al.*, 2016 technique was adjusted to determine the TPI. Before adding 500 μL 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% Na_2CO_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 Fe^{2+} Chelating Activity

With a few minor adjustments, the Hu *et al*., 2012 procedure was used for the Fe^{2+} chelation experiment (Karas *et al.*, 2015). In a nutshell, 1 mL of the sample was combined with a 2 mM $FeCl_2$ 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 $FeCl_3$ 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 $FeSO_4$, the data were presented as moles of Fe^{2+} per 100 grams of defatted weight. The wavelength of $7H_2O$ was 562 nm.

2.8 Calculation for $\%IC_{50}$ (Inhibition concentration 50 percent)

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

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

The IC_{50} 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_{50} 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_{50} 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 ecologicaleffect, little is known about their function as a source of bioactive compounds. We have demonstrated that marketexisting 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_{50} 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 µg/ml), and Silk worms(68.17 µg/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 $TEAC_{aq}$ (mmol TE/100 g). The TEAC (Troxol Equivalent Antioxidant Capacity) of water-soluble insect extracts for human consumption. The values are the mean ± SD of three replicates and are given as TEACaq (mmolTE/100 g of defatted sample or 100 mL). Trolox Equivalents, or TE.

Table 1: Radical Scavenging Activity (TEAC)

Concentration	**% IC_{50}**				
	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

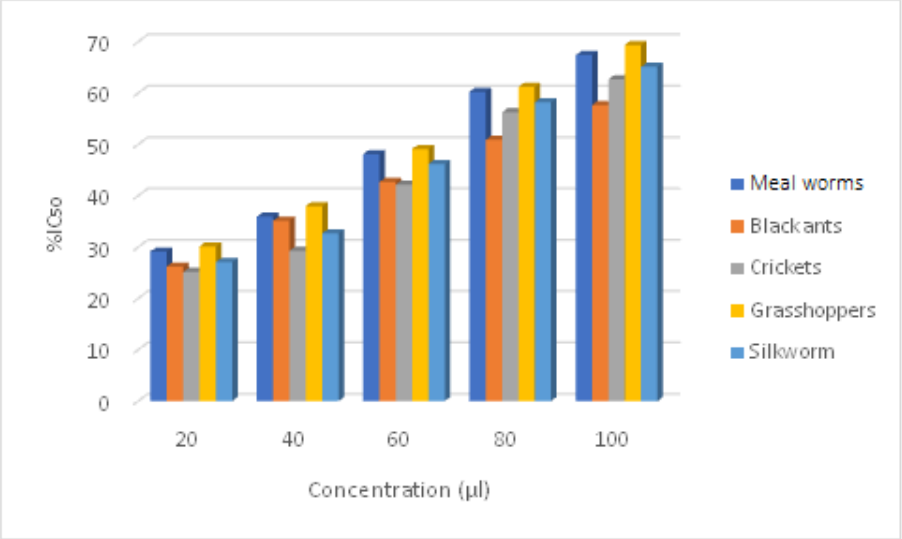

Figure 1: Radical Scavenging Activity (TEAC)

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 (Troxol Equivalent Antioxidant Capacity).

3.2 DPPH Radical Scavenging Activity Assay

The IC_{50} 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_{50}				
	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

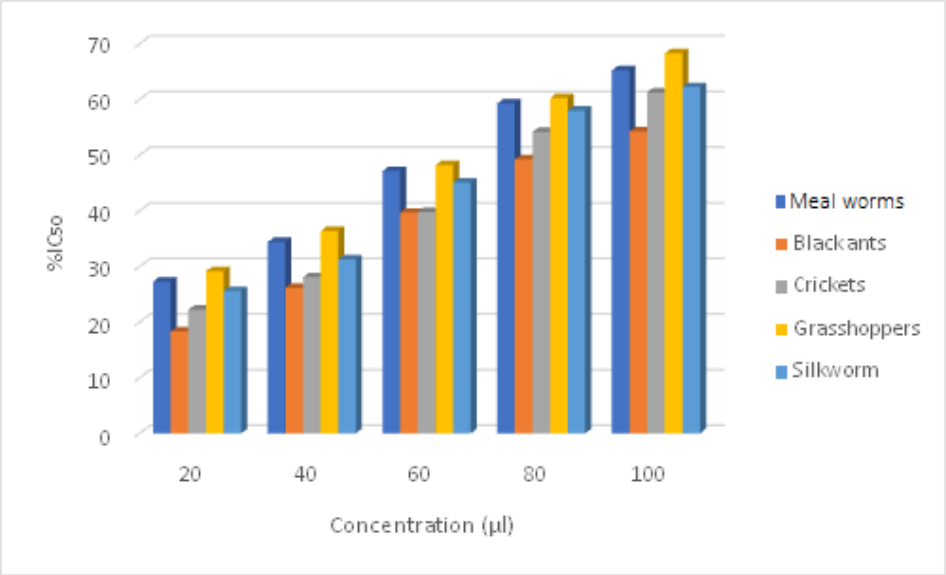

Figure 2: DPPH Radical Scavenging Activity Assay

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 TPIdemonstrates 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^{2+} Chelating Activity

Among all the hydrolysates examined, the various edible insect protein preparation hydrolysate demonstrated the highest ability to chelate Fe^{2+} ions. Meal worms (IC_{50} = 74.61 g/ml), Black ants (88.39 g/ml), Crickets (82.67 g/ml), Grasshoppers (67.16 g/ml), and Silk worms (IC_{50} = 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^{2+}.

Table 3: Determination of Fe^{2+} Chelating Activity

Concentration	% IC_{50}				
	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

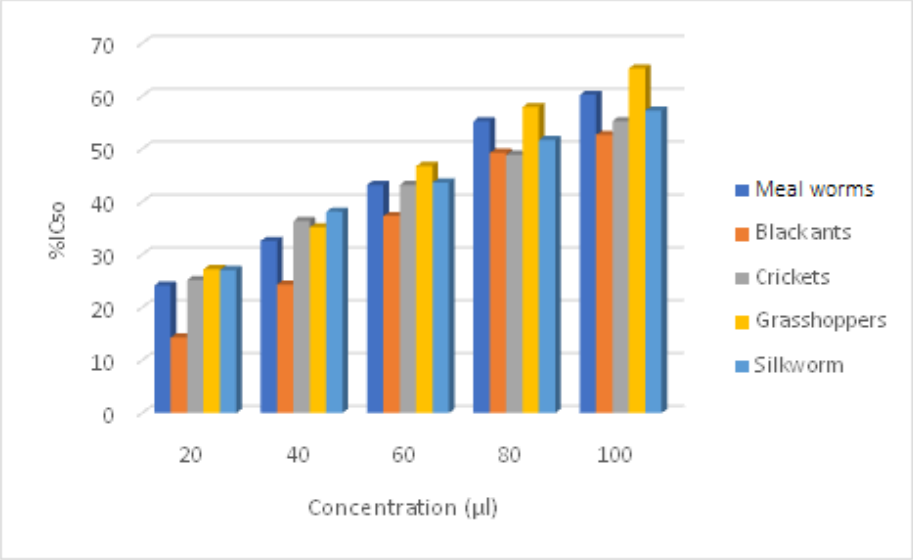

Figure 3: Determination of Fe^{2+} Chelating Activity

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 $Fe^{2+.}$ These results concur with those reported by Karas *et al.*, in 2015. With an 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 IC_{50} value for the edible insects is Meal worms (72.22 µg/ml), Black ants (88.40 µg/ml), Crickets (84.29 µg/ml), Grasshoppers (64.75 µg/ml), and Silk worms (76.94 µg/ml). The highest inhibition concentration shows in Grasshoppers and next to it are Meal worms.

Table 4: Ferric Reducing Antioxidant Power (FRAP)

Concentration	% 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

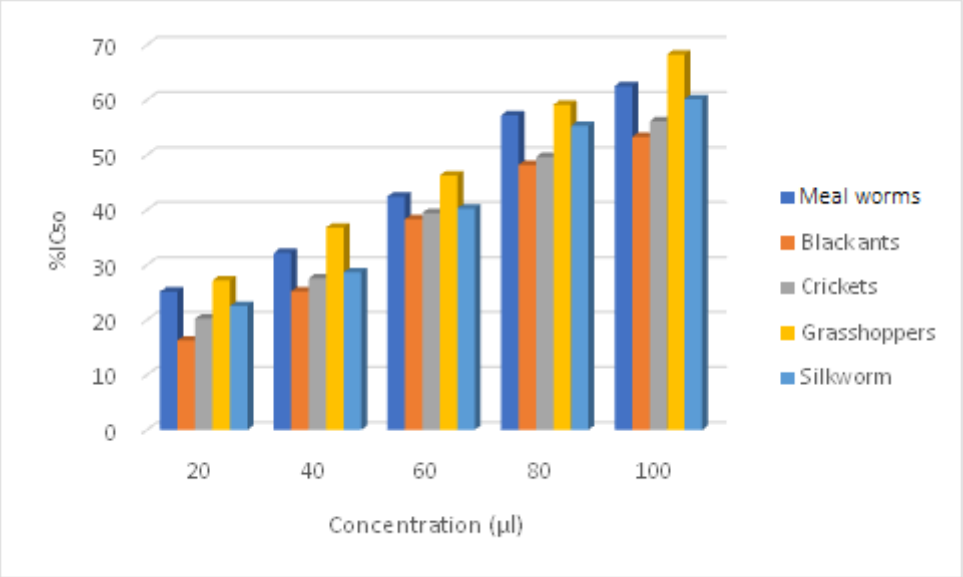

Figure 4: Ferric Reducing Antioxidant Power (FRAP)

The results highlight the importance of edible insects and invertebrates as maintainable original nutriments 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.

5. References

Ali, S. S., Kasoju, N., Luthra, A., Singh, A., Sharanabasava, H., Sahu, A., & Bora, U. (2008). Indian medicinal herbs as sources of antioxidants. *Food Research International*, *41*(1), 1–15. https://doi.org/10.1016/j.foodres.2007.10.001

Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytical Biochemistry*, *239*(1), 70–76. https://doi.org/10.1006/abio.1996.0292

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology*, *28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Carrasco-Castilla, J., Hernández-Álvarez, A. J., Jiménez-Martínez, C., Jacinto-Hernández, C., Alaiz, M., Girón-Calle, J., Vioque, J., &Dávila-Ortiz, G. (2012). Antioxidant and metal chelating activities of *Phaseolus vulgaris* L. var. Jamapa protein isolates, phaseolin, and lectin hydrolysates. *Food Chemistry*, *131*(4), 1157–1164. https://doi.org/10.1016/j.foodchem.2011.09.084

Castro-López, C., Santiago-López, L., Vallejo-Cordoba, B., González-Córdova, A. F., Liceaga, A. M., García, H. S., & Hernández-Mendoza, A. (2020). An insight into

fermented edible insects: A global perspective and prospective. *Food Research International*, *137*, 109750. https://doi.org/10.1016/j.foodres.2020.109750

Chakravorty, J., Ghosh, S., & Meyer-Rochow, V. B. (2011). Practices of entomophagy and entomotherapy by members of the Nyishi and Galo tribes, two ethnic groups of the state of Arunachal Pradesh (North-East India). *Journal of Ethnobiology and Ethnomedicine*, *7*, 5. https://doi.org/10.1186/1746-4269-7-5

Costa-Neto, E. M. (2005). Entomotherapy, or the medicinal use of insects. *Journal of Ethnobiology*, *25*(1), 93–114. https://doi.org/10.2993/0278-0771(2005)25[93:EOTMUO]2.0.CO;2

Dutta, P., Dey, T., Dihingia, A., Manna, P., & Kalita, J. (2017). Antioxidant and glucose metabolizing potential of edible insect, *Brachytrupesorientalis* via modulating Nrf2/AMPK/Glut4 signaling pathway. *Biomedicine and Pharmacotherapy*, *95*, 556–563. https://doi.org/10.1016/j.biopha.2017.08.094

Fu, Y., Young, J. F., &Therkildsen, M. (2017). Bioactive peptides in beef: Endogenous generation through postmortem aging. *Meat Science*, *123*, 134–142. https://doi.org/10.1016/j.meatsci.2016.09.015

Gerber, P. J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J. et al. (2013). Tackling climate change through livestock. A global assessment of emissions and mitigation opportunities. *Food and Agriculture Organizations of the United States*.

Giacintucci, V., Di Mattia, C., Sacchetti, G., Neri, L., &Pittia, P. (2016). Role of olive oil phenolics in physical properties and stability of mayonnaise-like emulsions. *Food Chemistry*, *213*, 369–377. https://doi.org/10.1016/j.foodchem.2016.06.095

Govorushko, S. (2019). Global status of insects as food and feed source: A review. *Trends in Food Science and Technology*, *91*, 436–445. https://doi.org/10.1016/j.tifs.2019.07.032

Hall, F., Johnson, P. E., & Liceaga, A. (2018). Effect of enzymatic hydrolysis on bioactive properties and allergenicity of cricket (Gryllodessigillatus) protein. *Food Chemistry*, *262*, 39–47. https://doi.org/10.1016/j.foodchem.2018.04.058

Hu, L., Song, R., & Gu, Z. (2012). An antioxidant peptide produced by autolysis reactions from wheat germ. *African Journal of Biotechnology*, *11*, 3640–3648.

Imathiu, S. (2020). Benefits and food safety concerns associated with the consumption of edible insects. *NFS Journal*, *18*, 1–11. https://doi.org/10.1016/j.nfs.2019.11.002

Jantzen da Silva Lucas, A., Menegon de Oliveira, L., da Rocha, M., & Prentice, C. (2020). Edible insects: An alternative of nutritional, functional, and bioactive compounds. *Food Chemistry*, *311*, 126022. https://doi.org/10.1016/j.foodchem.2019.126022

Karaś, M., Baraniak, B., Rybczyńska, K., Gmiński, J., Gaweł-Bęben, K., &Jakubczyk, A. (2015). The influence of heat treatment of chickpea seeds on antioxidant and fibroblast growth-stimulating activity of peptide fractions obtained from proteins digested under simulated gastrointestinal conditions. *International Journal of Food Science and Technology*, *50*(9), 2097–2103. https://doi.org/10.1111/ijfs.12872

Lettieri-Barbato, D., Tomei, F., Sancini, A., Morabito, G., & Serafini, M. (2013). Effect of plant foods and beverages on plasma non-enzymatic antioxidant capacity in human subjects: A meta-analysis. *British Journal of Nutrition*, *109*(9), 1544–1556. https://doi.org/10.1017/S0007114513000263

Magrone, T., Perez de Heredia, F., Jirillo, E., Morabito, G., Marcos, A., & Serafini, M. (2013). Functional foods and nutraceuticals as therapeutic tools for the treatment of diet-related diseases. *Canadian Journal of Physiology and Pharmacology*, *91*(6), 387–396. https://doi.org/10.1139/cjpp-2012-0307

Mason, J. B., Black, R., Booth, S. L., Brentano, A., Broadbent, B., Connolly, P., Finley, J., Goldin, J., Griffin, T., Hagen, K., Lesnik, J., Lewis, G., Pan, Z., Ramos, J. M., Ranalli, M., Rojas, G., Shockley, M., Stull, V. J., &Swietlik, D. (2018). Fostering strategies to expand the consumption of edible insects: The value of a tripartite coalition between academia, industry, and government. *Current Developments in Nutrition*, *2*(8), nzy056. https://doi.org/10.1093/cdn/nzy056

Mishyna, M., Martinez, J. I., Chen, J., & Benjamin, O. (2019). Extraction, characterization, and functional properties of soluble proteins from edible grasshopper (Schistocerca gregaria) and honey bee (Apis mellifera). *Food Research International*, *116*, 697–706. https://doi.org/10.1016/j.foodres.2018.08.098

Nongonierma, A. B., & Fitzgerald, R. J. (2017). Unlocking the biological potential of proteins from edible insects through enzymatic hydrolysis: A review. *Innovative Food Science and Emerging Technologies*, *43*, 239–252. https://doi.org/10.1016/j.ifset.2017.08.014

Oonincx, D. G., van Itterbeeck, J., Heetkamp, M. J., van den Brand, H., van Loon, J. J., & van Huis, A. (2010). An exploration of greenhouse gas and ammonia production by insect species suitable for animal or human consumption. *PLOS ONE*, *5*(12), e14445. https://doi.org/10.1371/journal.pone.0014445

Pimentel, D., & Pimentel, M. (2003). Sustainability of meat-based and plant-based diets and the environment. *American Journal of Clinical Nutrition*, *78*(3)(Suppl.), 660S–663S. https://doi.org/10.1093/ajcn/78.3.660S

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9–10), 1231–1237. https://doi.org/10.1016/s0891-5849(98)00315-3

Serafini, M., Bugianesi, R., Maiani, G., Valtuena, S., De Santis, S., & Crozier, A. (2003). Plasma antioxidants from chocolate. *Nature*, *424*(6952), 1013. https://doi.org/10.1038/4241013a

Serafini, M., Miglio, C., Peluso, I., &Petrosino, T. (2011). Modulation of plasma non-enzymatic antioxidant capacity (NEAC) by plant foods: The role of polyphenols. *Current Topics in Medicinal Chemistry*, *11*(14), 1821–1846. https://doi.org/10.2174/156802611796235125

Sethilkumar, N., Barthakur, N. D., & Rao, L. M. (2008). Bioprospecting concerning medicinal insects and tribes in India: An overview. *Indian For.*, *132*, 1575–1591.

Stadtman, E. R. (2006). Protein oxidation and aging. *Free Radical Research*, *40*(12), 1250–1258. https://doi.org/10.1080/10715760600918142

Stull, V. J., Finer, E., Bergmans, R. S., Febvre, H. P., Longhurst, C., Manter, D. K., Patz, J. A., & Weir, T. L. (2018). Impact of edible cricket consumption on gut microbiota in healthy adults, a double-blind, randomized crossover trial. *Scientific Reports*, *8*(1), 10762. https://doi.org/10.1038/s41598-018-29032-2

Tang, C., Yang, D., Liao, H., Liu, C., Wei, L., & Li, F. Edible insects as a food source: A review. Food Prod. Process. Nutricao 2019, *1*, 8–10.

Tang, Y., Debnath, T., Choi, E. J., Kim, Y. W., Ryu, J. P., Jang, S., Chung, S. U., Choi, Y. J., & Kim, E. K. (2018). Changes in the amino acid profiles and free radical scavenging activities of *Tenebrio molitor* larvae following enzymatic hydrolysis. *PLOS ONE*, *13*(5), e0196218. https://doi.org/10.1371/journal.pone.0196218

Tao, M., Wang, C., Liao, D., Liu, H., Zhao, Z., & Zhao, Z. (2017). Purification, modification and inhibition mechanism of angiotensin I-converting enzyme inhibitory peptide from Silk worms pupa (Bombyx mori) protein hydrolysate. *Process Biochemistry*, *54*, 172–179. https://doi.org/10.1016/j.procbio.2016.12.022

Van Huis, A., &Oonincx, D. G. A. B. (2017). The environmental sustainability of insects as food and feed. A review. *Agronomy for Sustainable Development*, *37*(5), 43. https://doi.org/10.1007/s13593-017-0452-8

Van Huis, A., Van Itterbeek, J., Klunder, H., Mertens, E., Halloran, A., Muir, G. et al. (2013). *Edible insects. Prospects for food and feed security*, *171* (pp. 60–64). Food and Agriculture Organization of the United Nations.

Vercruysse, L., Smagghe, G., Beckers, T., & Van Camp, J. V. (2009). Antioxidative and ACE inhibitory activities in enzymatic hydrolysates of the cotton leafworm, Spodoptera littoralis. *Food Chemistry*, *114*(1), 38–43. https://doi.org/10.1016/j.foodchem.2008.09.011

Wu, Q. Y., Jia, J. Q., Tan, G. X., Xu, J. L., & Gui, Z. Z. (2011). Physicochemical properties of Silk worms larvae protein isolate and gastrointestinal hydrolysate bioactivities. *African Journal of Biotechnology*, *10*, 6145–6153.

You, L., Zheng, L., Regenstein, J. M., Zhao, M., & Liu, D. (2012). Effect of thermal treatment on the characteristic properties of loach peptide. *International Journal of Food Science and Technology*, *47*(12), 2574–2581. https://doi.org/10.1111/j.1365-2621.2012.03136.x

Zhu, L. J., Chen, J., Tang, X. Y., & Xiong, Y. L. (2008). Reducing, radical scavenging, and chelation properties of in vitro digests of alcalase-treated zein hydrolysate. *Journal of Agricultural and Food Chemistry*, *56*(8), 2714–2721. https://doi.org/10.1021/jf703697e

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. https://doi.org/10.3390/nu9090970

Zielińska, E., Karaś, M., &Jakubczyk, A. (2017). Antioxidant activity of predigested protein obtained from a range of farmed edible insects. *International Journal of Food Science and Technology*, *52*(2), 306–312. https://doi.org/10.1111/ijfs.13282