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LC-MS/MS Targeted Amino Acid Profiling of Edible Beetle Anomala sp. and Its Host Plants

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Abstract

Anomala sp. is a green coleopteran beetle that feeds on the leaves of various fruit trees. It is one of the most preferred and abundantly found edible insects in Arunachal Pradesh, India. People from different countries consume it due to its nutritional and therapeutic value, both in cooked and uncooked form. This study aimed to assess the amino acid content in this edible insect and its host plant, which is still lagging. The method involves targeted metabolomic analysis using LC–MS/MS, producing reliable and reproducible data for quantifying free amino acids using deuterated internal standards without derivatization. Results showed that 0.57% and 0.36% of essential amino acids contributed to the total amino acids content in beetle and host plant's leaf, respectively, which does not meet the FAO requirement. Still, it has a higher content than related edible scarab beetle analyzed by other techniques. Amino acid content is significantly higher in edible beetle than in the host plant's leaf ($P < 0.001$).

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Introduction

Anomala beetle (Coleoptera: Scarabaeidae) is an edible leaf chafer beetle species, commonly known as 'Jojer' in Arunachal Pradesh.[1] This species is a polyphagous insect of major economic importance, seasonally available in India.[2] Its leaf-defoliating adult is a widely preferred edible beetle among tribal people of Arunachal Pradesh and the Northeastern region of India in general. Eating insects is a cultural and traditional practice found worldwide.[3,4] Its propagation is projected to have an immensely positive impact shortly as sustainable food or feed alternatives since population growth and resource depletion are imminent.[5]

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Assessing any food's nutritional and antinutritional composition is a prerequisite to ensure its quality and safety issues. Current trends in entomophagy research are widely focused on the assessment of this aforesaid important perspective. Among these nutrient contents, amino acids are mostly quantified in RP-HPLC (reversed-phase high-performance liquid chromatography) and GCMS (Gas chromatography-mass spectrophotometry) using derivatization method with ortho phthaldialdehyde and 9-fluorenyl methyl chloroformate.[6,7] These derivatization procedures are laborious and fail to analyze some amino acids though it gives a good resolution.[8] In our study, we used LCMS (Liquid chromatography-mass spectrophotometry) technique which allows amino acid analysis without the derivatization method.

Besides carbohydrates, protein is considered the second most abundant nutrient in food staples. and quantification of its composition is essential to evaluate its nutritional values. Amino acids are the building unit of proteins, so the quality and quantity of amino acids in any food staple define the characteristics feature of its protein quality.[9] In addition to its importance in the nutritional aspect, various researchers also widely explore free amino acids due to their important role in organoleptic functions, as a precursor of secondary metabolites, and in osmotolerance in higher plants.[10,11,12]

This present study aimed to determine the presence, and the patterns of the free amino acid profiles in the edible insect *Anomala* beetle consumed raw by the tribal population and its host plant leaves which are usually not considered edible. It can provide an important view into entomophagy practice concerning an amino acid in the food consumption and patterns of its distribution in edible beetle and plant they feed upon.

Materials and Methods

Insect Specimen and its Host Plant

Anomala beetle and host plant *Castanopsis indica* leaves were collected from the Old Ziro geographical locality (27°34'19.9" N and 93°48'19.0" E) of Arunachal Pradesh, India, in June 2021. This beetle is harvested by hand picking or shaking down in the net, which is spread on the ground, and collected. The Zoological Survey of India, Kolkata, and Botanical Survey of India, Itanagar, respectively, confirmed insect specimens and host plant identification.

Sample Preparation

200mg of the harvested samples were flash-frozen in liquid nitrogen and grounded properly. Each homogenized sample was extracted with 1 ml of 80% methanol followed by centrifugation at 4 C. The supernatant was diluted in water (1:20) [add 10µl of supernatant to 190 µl of water). was passed through a 0.2-micron syringe filter. 40 µl of the diluted supernatant was mixed with 360µl of label amino acid internal standard. This mix solution is kept at -20°C until analyzed in LC-MS/MS.

LC-MS/MS Analysis of Amino Acids

Using water that contained the 13C, 15 N-labelled algal amino acid mix (Cambridge Isotope Laboratories, Inc., USA) at a concentration of 10 g per ml, the methanolic extracts from insect and plant samples were diluted in a ratio of 1:20 (v:v). Amino acids in these diluted extracts were subsequently analyzed in LC-MS/MS Sciex QTRAP 6500+ triple-quadruple-trap MS/MS. The analysis process was revised from the protocols described in metabolomic research.[13,14,15] The amino acid was separated with a Zorbax Eclipse XDB-C18 column (50 × 4.6 mm, 1.8 µm, Agilent Technologies). The mobile phase comprised solvent A (water, 0.1% formic acid) and solvent B (acetonitrile, 0.1% formic acid). The flow rate was kept at 1.1 ml per min with the elution profile ran as follows: 0-1 min, 3% B; 1-3.8 min, 3-50% B; 3.8-3.9 min 50-100% B, 3.9-5 min, 100%B; 5-5.1 min, 100-3% B, 5.1-7 min, 100% B. The column was kept at a constant temperature of 27°C. The analyte parent ion and product ion were monitored using the mass spectrometer in positive ionization mode (MRM modus) for detection.[13] Both Q1 and Q3 quadruple were maintained at unit resolution. For data acquisition and processing of data, Analyst 1.5 software (Sciex) was used. The respective 13C and 15N-labeled amino acid internal standards were used to quantify individual amino acids in the sample.

Results and Discussion

LCMS/MS free amino acid analysis of an edible *Anomala* beetle showed the presence of 17 amino acids with 8 essential and 9 non-essential amino acids, and its host plant *C. indica* has 16 amino acids with 7 essential and 9 non-essential amino

acids (Table 1). Unpaired t-test showed no significant difference in the mean of the amino acid content in the beetle and host plant's leaf (df= 32, P>0.05). The quantity of each amino acid in the beetle was much higher than in its host plant leaves (P< 0.001). The essential amino acid methionine is also absent in the host plant sample. Non-essential amino acids such as aspartic acid, asparagine, arginine, and glutamic acids are demonstrated at a very high level in this beetle, and in the leaf, aspartic acid and asparagine are the highest.

Table 1: Amino acid types and content in *Anomala sp.* and its host plant of *C. indica* as quantitated by LC–MS/MS

Sl. No.	Amino acid	Type	Content µmol/mg±SD	
			Anomala sp.	***C. indica***
1.	Alanine	Non-Essential	32.504±22.936	0.067±0.017
2.	Serine	Non-Essential	7.028±4.930	0.056±0.009
3.	Valine	Essential	5.054±3.571	0.004±0.000
4.	Proline	Non-Essential	8.676±6.115	0.028±0.016
5.	Threonine	Essential	7.455±5.255	0.023±0.001
6.	Isoleucine	Essential	2.227±1.573	0.002±0.000
7.	Histidine	Essential	4.268±2.983	0.049±0.001
8.	Phenylalanine	Essential	2.098±1.477	0.009±0.002
9.	Arginine	Non-Essential	2.691±1.898	0.007±0.002
10.	Tyrosine	Non-Essential	1.055±0.740	0.008±0.004
11.	Tryptophan	Essential	0.302±0.213	0.001±0.002
12.	Glutamine	Non-Essential	10.468±7.353	0.069±0.016
13.	Lysine	Essential	7.193±5.059	0.038±0.010
14.	Glutamic acid	Non-Essential	25.783±18.200	0.044±0.008
15.	Asparagine	Non-Essential	901.408±625.027	17.486±5.946
16.	Aspartic acid	Non-Essential	4270.423±3007.301	17.457±2.172
17.	Methionine	Essential	1.212±0.857	0

The edible scarab beetle and the host plant sample undertaken in our study do not meet the FAO-provided requirements of 40% essential amino acids and 0.6 ratios of essential to non-essential amino acids (FAO/WHO 1973).[16] Similar results were shown in the study of a related edible beetle, *Holotrichia sp*., in Thailand.[17] In our result, only a meager 0.57% and 0.36% of essential amino acids are contributed to total amino acids in beetle and host plant's leaf, respectively. In general, leaf amino acids, mostly non-essential, that are associated with primary carbon metabolism and nitrogen assimilation are found in high concentrations, and essential amino acids in the human diet are considered minor and found less abundant.[18] A similar pattern is observed in the beetle. Despite this fact, the practice of consuming this beetle among tribal people is unlikely to decline, mainly due to its bioavailability and delicacy. The overall protein and amino acid content percentages are usually higher in edible insects than in plants.[19,20,21] However, the digestibility of insect protein is quite variable due to its exoskeleton.[22] Nevertheless, an insect diet is a crucial practice for many people throughout the world while many may consider its consumption as repulsive but the fact that lobster which was considered a water cockroach in the past is a luxury food item now. Research has shown that edible insects are not only a source of protein and amino acids, but decent sources of fatty acids, minerals, and vitamins.[23] The edible scarab beetles are known to be a good source of magnesium, iron, and zinc.[17]

Conclusion

Our study focuses on the nutraceutical competence of this edible insect and host plant with respect to

amino acid content, while exploring the efficiency of the recently developed underivatized targeted metabolomic LCMS/MS analytical technique in this application. This study concludes that LCMS/MS analysis of 17 free amino acids in the edible insect *Anomala sp*. and its host plant leaves provided a reliable amino acid content profile. The analysis of protein composition is always crucial for the quality assurance of insect diet, which defines the essential amino acid to non-essential amino acids ratio as recommended by FAO/WHO 1973.[19] It showed that this edible beetle does not meet the FAO recommendation nor does its host plant. However, the amino acid content is much higher than reported studies on other edible scarab beetles conducted with different chromatographic techniques. Derivatization is usually required to give reliable and reproducible results for quantifying amino acids. However, this process is usually laborious, and trivial slipups can have adverse effects on its quantification and thus hampering its analysis. So, this LCMS-targeted metabolomic analysis method using deuterated internal standards for each amino acid is quite efficient and sidelines the derivatization process. The amino acid quantity and composition are found to be higher in the edible beetle than in its non-edible host plant's leaf, and the essential amino acid methionine is also absent in the host leaf. However, since the present result assessed only amino acid contents in this edible beetle, more studies on the various components, such as fatty acids, minerals, vitamins etc., are necessary for a proper implication on nutraceutical value and project its acceptability as a quality human palatable food. Though the development of the superlative amino acid analytical method continues to be a challenge, this underivatized LCMS/MS technique is widely accepted for the quantification of all 20 proteinogenic amino acids. Nevertheless, comparative quantification of amino acids using various chromatographic techniques is suggested to validate the efficiency of each technique in both time, sensitivity and comprehensiveness.

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Conflict of Interest

The authors declare no conflict of interest.

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