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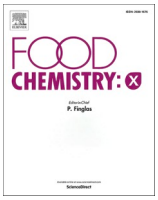

Chemical composition and nutritional profile of cicada (*Meimuna opalifera* Walker) at different developmental stages: Implications for functional food applications

Hua Li [a], Theeraphan Chumroenphat [b,d], Apichaya Bunyatratchata [b,c], Parinya Boonarsa [b,c], Colin Wrigley [e], Sirithon Siriamornpun [b,c,*]

[a] *Department of Cuisine and Nutrition, Yangzhou University, Yangzhou, Jiangsu Province 225127, China*
[b] *Research Unit of Thai Food Innovation (TFI), Mahasarakham University, Kantarawichai, Maha Sarakham 44150, Thailand*
[c] *Department of Food Technology and Nutrition, Faculty of Technology, Mahasarakham University, Kantarawichai, Maha Sarakham 44150, Thailand*
[d] *Aesthetic Sciences and Health Program, Faculty of Thai Traditional and Alternative Medicine, Ubon Ratchathani Rajabhat University, Ubon Ratchathani 34000, Thailand*
[e] *Center for Crop Science, Queensland Alliance for Agriculture and Food Innovation, University of Queensland, Brisbane 4067, Australia*

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ABSTRACT

This investigation explored chemical changes in cicadas during their developmental stages (nymph, late nymph, and adult). Tocopherols (α, δ, γ) were found at a total content of 13.7 mg/g, while γ-oryzanol was observed at 2.6 mg/g, with nymphs having the highest levels, followed by late nymphs and adults. Essential amino acids increased progressively with maturation, with methionine being the predominant amino acid in all samples. The index of essential amino acids in each tissue was as follows: adult (0.36), late nymph (0.33), and nymph (0.12). Eicosapentaenoic acid concentrations varied from 230 mg/100 g in adults to 880 mg/100 g in nymphs. Protein analysis using the Protein Simple Jess system revealed a molecular weight distribution ranging from 10 to 75 kDa, accounting for approximately 70 % of the total protein content. These findings offer valuable insights for incorporating cicadas as functional food ingredients, diversifying food product formulations.

1. Introduction

In recent years, the nutritional value and eco-friendliness of edible insects have captured the attention of researchers and consumers globally. Remarkably, more than 1900 insect species have been recognized as potential sources of human food at various stages of their life cycle, as highlighted by Kinyuru, Mogendi, Riwa, and Ndung'u (2015). Regions such as Africa, Asia, and Latin America, where livestock resources may be limited or alternative protein sources are scarce, have a rich history of incorporating a diverse range of insect species into their traditional diets (Raksakantong, Meeso, Kubola, & Siriamornpun, 2010). In Thailand, edible insects have a long culinary history, especially in rural areas, but have recently gained popularity among urbanites and foreign tourists. Bangkok, for example, has farmer's markets and supermarkets where more than one hundred insect species are collected and sold in abundance (Pal & Roy, 2014).

Consumption of these edible insects varies depending on seasonal availability, with a myriad of species being highly favored, including cicadas, crickets, wasps, locusts, beetles, bees, ants, caterpillars, grasshoppers, scale insects, true bugs, termites, leaf and plant hoppers, dragonflies, and flies (Ishara et al., 2022; Pal & Roy, 2014). Cicada (*Meimuna opalifera* Walker), an insect species inhabiting terrestrial environments, possesses distinct morphological attributes characterized by robust bodies, broad heads, transparent membranous wings, and prominent compound eyes. This insect undergoes a life cycle encompassing three stages: eggs, nymphs, and adults, exhibits notable abundance during the rainy season, and holds a significant position within the culinary customs of Southeast Asia, with particular prominence in the northeastern region of Thailand. The female cicadas, renowned for their substantial flesh content, have garnered heightened popularity among consumers. Furthermore, cicadas have been employed in traditional Chinese medicine. The expansive family Cicadidae encompasses approximately 3100 species, as reported by Zhang, Wang, Billen, and Wei (2021). While cicadas have been recognized for their potential as a

* Corresponding author.
E-mail address: sirithons@hotmail.com (S. Siriamornpun).

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food source, offering diverse biological effects such as antioxidant, antitumor, and immunomodulatory activities (Tian et al., 2021), research exploring their chemical composition remains limited. Previous investigations have focused on the presence of 14 inorganic elements in cicadas (Lehnert, Reiter, Smith, & Kritsky, 2019) as well as the characterization of fatty acids, proteins, lipids, carbohydrates, and fiber (Raksakantong et al., 2010). To the best of our knowledge, the nutrition in cicada at different growth stages has not been studied yet. In this study, we investigated the nutrition in cicada at various growth stages, namely, nymph, late nymph, and adult. The contents of chemical components in terms of phenolic acid, flavonoid, γ-oryzanol, α-, δ-, and γ-tocopherols, amino acid, and fatty acid were investigated. This research should serve as a helpful foundation for future study on the processing and production of functional food products, providing valuable information.

2. Materials and methods

2.1. Chemicals and reagents

All standards for chromatographic analysis were purchased from Sigma-Aldrich Co. (St. Louis, USA). Thirteen standards of phenolic acids were gallic acid, protocatechuic acid, chlorogenic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, vanillin, *p*-coumaric acid, ferulic acid, syringic acid, sinapic acid, cinamic acid, and genistic acid. Five standards of flavonoids included rutin, kaempferol, quercetin, apigenin, and myricetin. Twenty and sixteen standard compounds were used to determine amino acids and fatty acids, respectively. All chemicals used except for the chromatography analysis were analytical grade from Merck (Darmstadt, Germany).

2.2. Sample preparation

Cicadas at different growth stages were obtained from a local market in Sakon Nakhon, Thailand, and fasted for about 48 h to rid their digestive tracts of any leftover meals. One kilogram of each sample was freeze-dried using a Scanvac CoolSafe (100–9 Pro, LaboGene ApS, Denmark) and then ground in a laboratory grinder and placed at − 20 °C until used.

2.3. Determination of proximate composition

According to AOAC official method (2000), the crude protein (981.10), crude fat (960.39), crude fiber (934.01) and ash (920.153) contents were measured. The carbohydrate content was calculated as [100 – (%crude protein + %crude fat + %crude fibre +%ash)].

2.4. HPLC determination of phenolic acids and flavonoids

Phenolic acids and flavonoids were extracted as described previously by Chumroenphat, Somboonwatthanakul, Saensouk, and Siriamornpun (2021) and analyzed by HPLC (Shimadzu, Kyoto, Japan) following the protocol of Kubola, Siriamornpun, and Meeso (2011). Briefly, samples (1.0 g) were extracted at 37 °C for 12 h with 20 mL of HCl/methanol (1:100, v/v) solution while being shaken at 150 rpm in the dark. Following filtering, the pellet performed a second extraction, and the mixed filtrates were dried under vacuum at 40 °C. Prior to HPLC analysis, the residue was redissolved in 5 mL of methanol/water (1:1, v/v) and filtered through a 0.45 μm nylon membrane. The phenolics were separated by an InertSustain® C18 column (250 mm × 4.6 mm, 5 μm; GL Sciences Inc., Tokyo, Japan). The wavelengths for detecting hydroxybenzoic acids, hydroxycinnamic acids, and flavonoids were 280, 320, and 370 nm, respectively. The phenolics in the extracted samples were identified according to the retention time and spectrum of each standard.

2.5. Extraction and determination of γ-oryzanol and α-, δ-, and γ-tocopherols

The solvent extraction method employed for the cicada sample followed a modified version of the technique described by Chen and Bergman (2005). One gram of the sample was vigorously mixed with acetone:water (1:10, v/v) using a vortex, and subsequently subjected to centrifugation at 2,500 × g for a duration of 20 min. The resulting residue underwent two additional extractions using the same procedure. The combined supernatants were then evaporated to dryness under a stream of nitrogen gas. The analysis of γ-oryzanol and tocopherols contents was conducted using an RP-HPLC system (Shimadzu, Kyoto, Japan) equipped with a photodiode-array detector. Acetonitrile/methanol served as the mobile phase, with the wavelengths of 325 nm and 292 nm utilized for the detection of γ-oryzanol and tocopherols, respectively.

2.6. Determination of amino-acid content

The powder of the freeze-dried cicada material was defatted as follows: 5 g of powder was dispersed in 50 mL of *n*-hexane, stirred for 30 min and centrifuged (Kim et al., 2020). The residue was further defatted by *n*-hexane for three times. After placed in a fume hood overnight, the defatted powder without residual hexane was mixed with 5 mL of 6 M HC1 and put at 110 ℃ for 15 h in a hot-air oven (FED 115, WTB Binder, Germany) using the method provided by Ladrón de Guevara, Padilla, García, Pino, and Ramos-Elorduy (1995). After hydrolysis, the HC1 was removed by using a rotary evaporator (Buchi *R*-3, Vacuum Pump v-700, Switzerland) at 60 ℃. The dried hydrolysate dissolved in purified water (1:10, w/v) was centrifuged at 12000 × g for 10 min, and then the supernatant was filtered with a 0.22 μm nylon membrane before LC/MS/MS analysis. LC/MS/MS (LCMS-8030 triple quadrupole mass spectrometer, Shimadzu, Kyoto, Japan) was conducted by electrospray ionization, together with a HPLC system. Amino acids were gradiently eluted on an InertSustain® C18 column (2.1 mm × 150 mm, 3 μm). The LC/MS/MS condition followed the method of Chumroenphat et al. (2021). The results were displayed as milligram of amino acids in 1.0 g sample on dry basis. The essential amino acid index (EAAI) was calculated according to the formula below (WHO/FAO/UNU, 2007).

$$\mathrm{EAAI} = \sqrt[10]{\frac{mg\ of\ lysine\ in\ 1\ g\ of\ test\ protein}{mg\ of\ lysine\ in\ 1\ g\ of\ reference\ protein}} x\ etc\ ::\ for\ the\ other\ 9\ essential\ amino\ acids$$

2.7. Determination of fatty-acid content

Fatty-acid methyl esters (FAMEs) were derived from the total lipid extracts through transesterification using a methanol solution of H_2SO_4 (0.9 M). The resulting FAMEs were subjected to quantitative analysis using a GC system (GC-2014, Shimadzu, Tokyo, Japan) equipped with a flame ionization detector and a fused silica capillary column (30 m × 0.25 mm, 25 μm; DB-Wax, USA). Identification of fatty acids was accomplished by comparing the retention time of standard FAMEs

Table 1
Proximate composition and γ-oryzanol and tocopherols contents of Thai cicada at different growth stages.

Parameter	Cicada		
	Nymph	Late nymph	Adult
Protein (g/100 g)	15.49 ± 0.07^{c}	56.71 ± 0.24^{a}	56.35 ± 0.09^{b}
Fat (g/100 g)	61.84 ± 0.59^{a}	11.01 ± 0.28^{c}	13.37 ± 0.07^{b}
Fiber (g/100 g)	8.03 ± 0.11^{c}	13.37 ± 0.11^{b}	16.87 ± 0.47^{a}
Ash (g/100 g)	1.96 ± 0.06^{c}	5.36 ± 0.05^{a}	5.03 ± 0.23^{b}
Carbohydrate (g/100 g)	12.67 ± 0.34^{b}	13.51 ± 0.36^{a}	8.38 ± 0.15^{c}
δ-tocopherol (μg/g)	5324.85 ± 42.65^{a}	106.62 ± 1.66^{b}	4.50 ± 0.85^{c}
γ-tocopherol (μg/g)	2119.42 ± 25.17^{a}	12.35 ± 0.47^{b}	ND
α-tocopherol (μg/g)	6269.70 ± 109.63^{a}	42.41 ± 1.82^{c}	158.05 ± 0.40^{b}
γ-oryzanol (μg/g)	2587.41 ± 108.15^{a}	ND	1.51 ± 0.04^{b}

Values are expressed as mean ± SD of triplicate measurements (n = 3). Means with different letters are significantly different at $p < 0.05$ within the same row in the parameter. ND: Not detected.

analyzed under the same method (Yang, Siriamornpun, & Li, 2006), while quantification was performed using the internal standard (nonadecanoic acid, C19:0) method.

2.8. Determination of molecular weights of protein components

2.8.1. Protein extraction procedure

Protein was extracted using the method of Kim et al. (2020). Briefly, 1 g of the defatted powder prepared as in 2.6 was homogenized in 10 mL of 0.02 % ascorbic acid and filtered through medical gauze. The filtrate was centrifuged at 10000 × g for 30 min. The obtained supernatant was used in the western analysis.

2.8.2. Evaluation of protein molecular weight using Jess simple western analysis

Capillary-based western analysis utilizing the Protein Simple Jess system (Protein Simple, CA, USA) determined protein MW. Briefly, cell lysates were diluted by sample buffer to 1 mg/mL and mixed with a master mix of 40 mM dithiothreitol, 1 × sample buffer, and 1 × fluorescence standard. After heating at 95 °C for 5 min, 3 μL of the denatured protein extract, 10 μL of each primary antibody (at 1:20 dilution), HRP-conjugated anti-mouse secondary antibodies, chemiluminescent substrate, and protein normalization solution were loaded into the assay plate wells. Anti-JAK2 and anti-STAT5 were the main antibodies. Biotinylated ladder cartridges provided MW standards (12–230 kDa) for each experiment. The Jess machine automated protein electrophoresis with the plate and capillaries. Capillaries were adjusted for protein loading. Compass (Protein Simple, CA, USA) assessed chemiluminescent processes.

2.9. FT-IR measurements

Nymph, late nymph and adult extracts (in powder form without any special preparation) were analyzed using an FT-IR instrument (Frontier) with an UATR accessory (Perkin Elmer, USA) equipped with a Diamond/KRS-5 crystal composite (1 bounce). Spectral data were achieved with 32 scans and a force gauge of 110 units. The covering region was in the range of 4000–400 cm^{-1} and the resolution was 4 cm^{-1}. A recorded background spectrum was automatically subtracted by the software.

2.10. Statistical analyses

All results were displayed as the mean ± one standard deviation (SD) of three replicates. One-way ANOVA and the least significant difference (LSD) test were used to analyze the data. Different letters among samples indicate significant difference ($p < 0.05$).

Table 2
Contents of phenolic acids and flavonoids in Thai cicada at different growth stages.

Parameter	Phenolic content (μg/g)		
	Nymph	Late nymph	Adult
Phenolic acid			
gallic acid	216.46 ± 0.50^{b}	90.82 ± 0.11^{a}	ND
protocatechuic acid	11.67 ± 0.14^{g}	6.15 ± 0.27^{f}	22.99 ± 0.75^{c}
p-hydroxybenzoic acid	49.77 ± 0.53^{e}	ND	ND
chlorogenic acid	57.96 ± 3.62^{d}	21.66 ± 0.37^{e}	10.20 ± 0.03^{d}
vanillic acid	21.30 ± 2.06^{f}	34.70 ± 0.41^{d}	30.66 ± 1.50^{b}
caffeic acid	ND	ND	ND
syringic acid	3.91 ± 0.17^{h}	57.08 ± 0.09^{b}	61.15 ± 0.14^{a}
vanillin	3.06 ± 0.35^{i}	5.46 ± 0.20^{g}	5.27 ± 0.27^{e}
p-coumaric acid	0.48 ± 0.02^{k}	0.21 ± 0.02^{j}	0.43 ± 0.03^{h}
ferulic acid	2.41 ± 0.06^{j}	ND	0.72 ± 0.04^{g}
sinapic acid	20.11 ± 0.43^{f}	1.79 ± 0.34^{i}	3.61 ± 0.23^{f}
cinnamic acid	603.94 ± 54.08^{a}	38.11 ± 1.82^{c}	ND
genistic acid	125.44 ± 16.88^{c}	4.56 ± 0.05^{h}	ND
total	1116.00 ± 39.34^{A}	261.00 ± 3.68^{B}	135.03 ± 2.99^{C}
Flavonoid			
rutin	12.32 ± 0.15^{e}	5.69 ± 0.13^{c}	ND
quercetin	112.43 ± 3.56^{c}	ND	ND
apigenin	91.39 ± 3.52^{d}	21.87 ± 1.29^{b}	15.68 ± 0.44^{c}
kaempferol	129.08 ± 6.83^{b}	ND	20.14 ± 0.10^{b}
myricetin	296.76 ± 10.95^{a}	95.68 ± 0.94^{a}	131.82 ± 3.44^{a}
total	641.76 ± 5.00^{A}	123.24 ± 0.47^{C}	167.64 ± 0.80^{B}

Values are expressed as mean ± SD of triplicate measurements (n = 3). Means with different lowercase letters and capital letters are significantly different at $p < 0.05$ within the same column and same row in the parameter, respectively. ND: Not detected.

Table 3
Amino-acid content in Thai cicada at different growth stages.

Parameter	Amino-acid content (mg/g)		
	Nymph	Late nymph	Adult
Essential			
arginine	8.01 ± 0.01^{b}	16.66 ± 0.56^{a}	17.27 ± 0.14^{a}
histidine	9.12 ± 0.34^{b}	24.12 ± 1.22^{a}	22.48 ± 1.01^{a}
isoleucine	4.56 ± 0.11^{c}	15.89 ± 0.03^{a}	15.25 ± 0.12^{b}
leucine	4.24 ± 0.05^{c}	13.85 ± 0.10^{a}	13.37 ± 0.16^{b}
lysine	2.63 ± 0.01^{b}	6.94 ± 0.23^{a}	6.68 ± 0.17^{a}
methionine	16.25 ± 0.42^{c}	71.74 ± 0.63^{b}	78.05 ± 1.16^{a}
phenylalanine	3.0 ± 0.02^{b}	13.98 ± 0.42^{a}	13.85 ± 0.15^{a}
threonine	2.70 ± 0.04^{c}	3.54 ± 0.35^{b}	3.94 ± 0.09^{a}
tryptophan	0.04 ± 0.00^{b}	0.04 ± 0.00^{b}	0.08 ± 0.00^{a}
valine	6.06 ± 0.14^{c}	19.91 ± 1.15^{a}	18.47 ± 0.19^{b}
sum-Essential	57.48 ± 0.53^{b}	187.57 ± 3.58^{a}	189.28 ± 2.04^{a}
Non-Essential			
alanine	3.43 ± 0.16^{c}	14.16 ± 0.23^{b}	14.80 ± 0.16^{a}
asparagine	ND	ND	ND
aspartic acid	6.78 ± 0.78^{b}	14.31 ± 0.52^{a}	14.42 ± 0.16^{a}
cysteine	0.07 ± 0.00^{c}	0.12 ± 0.01^{b}	0.17 ± 0.01^{a}
glutamine	2.76 ± 0.01^{c}	7.44 ± 0.08^{a}	7.09 ± 0.14^{b}
glutamic acid	6.68 ± 0.04^{b}	11.08 ± 0.37^{a}	11.56 ± 0.37^{a}
glycine	2.16 ± 0.02^{c}	4.61 ± 0.09^{a}	4.24 ± 0.17^{b}
proline	4.22 ± 0.15^{c}	9.81 ± 0.06^{a}	9.36 ± 0.09^{b}
serine	4.59 ± 0.09^{b}	7.97 ± 0.03^{a}	7.94 ± 0.21^{a}
tyrosine	22.23 ± 0.24^{c}	73.53 ± 0.81^{a}	62.61 ± 0.49^{b}
sum-non-Essential	52.95 ± 0.28^{c}	143.03 ± 1.93^{a}	132.18 ± 0.92^{b}
total amino acids	110.44 ± 0.37^{c}	330.60 ± 5.48^{a}	321.46 ± 2.58^{b}
EAAI	0.12 ± 0.01^{c}	0.33 ± 0.01^{b}	0.36 ± 0.01^{a}

Values are expressed as mean ± SD of triplicate measurements (n = 3). Means with different letters are significantly different at $p < 0.05$ within the same row of the parameter. ND: Not detected.

3. Results and discussion

3.1. Proximate composition

Table 1 presents the proximate composition of cicadas at various growth stages. Among the different stages, the nymphs exhibited the lowest levels of protein, fiber, and ash contents, whereas their fat content was the highest ($p < 0.05$). Conversely, the late nymphs and adults showed abundant protein content, surpassing 50 % and reaching approximately 3.7 times higher levels compared to the nymphs. This value is also higher than that of jumil (32 %), wasp larvae (38 %), and black soldier fly larvae (38 %) reported by Baigts-Allende et al. (2021), and comparable to that of house crickets (54 %) and mulberry silkworms (50 %) reported by Köhler, Kariuki, Lambert, and Biesalski (2019). In contrast, the nymphs displayed a high fat content, exceeding 60 % and representing approximately 5.6 and 4.6 times higher values than those observed in the late nymphs and adults, respectively. Additionally, the value is comparable to that of *Rhynchophorus phoenicis* (52–62 %), but much higher than that of *Tenebrio molitor* (21–38 %) and *Oryctes rhinoceros* (8–20 %) (Meyer-Rochow, Gahukar, Ghosh, & Jung, 2021). Similarly, higher protein content but lower fat content in parallel with the developmental stages was also observed in the grasshopper *Zonoceros variegatus* (Ademolu, Idowu, & Olatunde, 2010) and three Blattodea species, namely *Blaptica dubia*, *Blaberus discoidalis*, and *Blatta lateralis* (Kulma et al., 2016). This phenomenon may be partly attributed to the difference in lifestyle between nymphs and adults and the greater requirement for burning fat to meet the energy demand for adults than for nymphs (Meyer-Rochow, Gahukar, Ghosh, & Jung, 2021). In addition, the nutritional composition at different developmental stages may be related to the nutritional status of the host plants as well as the insects' life cycle physiologies affected by the season and climate (Meyer-Rochow et al., 2021).

3.2. Phenolic acid and flavonoid contents

The chemical composition of cicadas at different growth stages was investigated, and the results are summarized in Table 2. Notably, the phenolic acid and flavonoid contents were examined to gain insights into the composition dynamics. In terms of total phenolic acid content, the nymph stage exhibited the highest concentration compared to the late nymph and adult stages ($p < 0.05$). Further analysis of phenolic acids within the nymph samples revealed cinnamic acid as the most abundant compound. In contrast, gallic acid and syringic acid were the predominant phenolic acids identified in the late nymph and adult stages, respectively. It is noteworthy that *p*-hydroxybenzoic acid was exclusively detected in the nymph stage and was absent in the late nymph and adult stages, while caffeic acid was not detected in any of the samples. These findings suggest that the high levels of cinnamic and genistic acids present in nymphs hold economic potential for various applications. Furthermore, an examination of the flavonoid content at different growth stages revealed the highest total flavonoid content in the nymph stage ($p < 0.05$). Myricetin emerged as the predominant flavonoid across all stages. Interestingly, quercetin was exclusively detected in nymph tissue. Therefore, both the phenolic acid and flavonoid data must be collectively analyzed to comprehend the overall content.

The presence of phenolic acids and flavonoids in edible insects, including cicadas, may be related to the insects' absorption and metabolism of phenolic compounds derived from their dietary sources, as well as their ability to synthesize phenolics through the sclerotization process (Nino, Reddivari, Osorio, Kaplan, & Liceaga, 2021), which probably led to the variation in phenolic content among cicadas at different developmental stages. For example, in *Polyommatus icarus* adults, approximately 80 % of the flavonoids were found in the wings, while the remainder was distributed throughout the body. Musundire, Zvidzai, Chidewe, Samende, and Manditsera (2014) demonstrated the presence of phenolics in edible ground crickets (*Henicus whellani*) from Zimbabwe and quantified total phenolics (7.7 mg/g), tannins (0.17 mg/g), and flavonoids (15.5 mg/g), indicating the insect's capacity to absorb and sequester these compounds from plants. Insects, particularly those in the Lepidoptera order, possess the ability to metabolize dietary phenols and incorporate them into their body structures, which results in the selective uptake of certain flavonoids, such as kaempferol and quercetin, as observed in the studies reviewed by Giampieri et al. (2022). Moreover, phenolic acids have been reported to impact insect growth significantly. For instance, caffeic acid has been observed to increase oxidative stress and reduce immune responses in insects. Such effects can detrimentally impact the overall fitness of insects, notably impairing their growth and development, as seen in the study by Punia, Singh, Thakur, and Chauhan (2023) on *Spodoptera litura* larvae. This suggests that the specific presence of phenolic compounds in the nymph stage of cicadas could be related to these compounds' roles in affecting physiological functions like stress response, immunity, and developmental processes.

Although some work has been done on the total contents of several categories of phenolics, limited research has explored the individual phenolic acid and flavonoid contents in insects. This present study contributes novel insights into the phenolic acid and flavonoid profiles of cicadas at different growth stages, expanding our understanding of their chemical composition.

3.3. Tocopherols and γ-Oryzanol

Tocopherols, recognized as vitamin E, play a crucial role as antioxidants. Their primary biological function involves safeguarding polyunsaturated fatty acids (PUFAs) and other compounds present in cell membranes and low-density lipoproteins against oxidation caused by free radicals (Fogang Mba et al., 2017). In our analysis, we identified three tocopherol isomers, namely α-tocopherol, δ-tocopherol, and

γ-tocopherol, in all examined samples. The isomeric distribution of tocopherols in cicadas at different growth stages is presented in Table 1. Notably, α-tocopherol emerged as the most prevalent tocopherol in all stages of cicada development, followed by δ-tocopherol and γ-tocopherol. Our findings revealed that the nymph stage exhibited the highest tocopherol (δ-, γ-, α-) contents among the examined samples, with α-tocopherol reaching remarkable concentrations of up to 6270 μg/g ($p < 0.05$). These values considerably surpassed the contents observed in other sources such as *Rhynchophorus phoenicis* larvae (95 μg/g lipid) (Fogang Mba et al., 2017), mealworm *Tenebrio molitor* (45 μg/g lipid), and waxworm *Galleria mellonella* (758 μg/g lipid) (Barker, Fitzpatrick, & Dierenfeld, 1998). Moreover, the α-tocopherol content in cicadas surpassed those reported in crude rice bran oil (161 μg/g) (Pestana-Bauer, Zambiazi, Mendonça, Beneito-Cambra, & Ramis-Ramos, 2012), as well as in pumpkin (9 μg/g), barley (15 μg/g), quinoa (21 μg/g), chickpea (69 μg/g), and pea (104 μg/g) (Ryan, Galvin, O'Connor, Maguire, & O'Brien, 2007). These findings underscore the substantial tocopherol content in cicadas, particularly during the nymph stage, surpassing the levels observed in other comparable sources.

γ-Oryzanol primarily comprises esters of trans ferulic acid with phytosterols, encompassing sterols and triterpenic alcohols (Tuncel & Yılmaz, 2011). Extensive research has confirmed its antioxidative properties and association with reduced plasma and serum cholesterol levels, cholesterol absorption, and platelet aggregation (Thammapat, Meeso, & Siriamornpun, 2016). The comprehensive results pertaining to γ-oryzanol are summarized in Table 1. Interestingly, our findings distinctly highlight the nymph stage as the most abundant source of γ-oryzanol ($p < 0.05$), while the compound was not detected in the late nymph. Notably, the nymph exhibited an impressive γ-oryzanol content of 2,587 μg/g. Although this value surpasses the contents observed in purple rice varieties (398–753 μg/g) (Boonsit, Pongpiachan, Julsrigival, & Karladee, 2010), it falls short of the levels found in crude rice bran oil (12,400 μg/g) (Pestana-Bauer et al., 2012). Nonetheless, the cicada nymph emerges as a promising dietary source of both γ-oryzanol and tocopherols. To the best of our knowledge, this study represents the first report on the γ-oryzanol content and tocopherol composition of cicada at different growth stages. The remarkable levels of oryzanol and tocopherols found in cicada nymphs hold potential for utilizing raw insects in the production of derived products, contributing to the advancement of insect-based food processing.

3.4. Amino-acid content

The assessment of protein quality relies on the analysis of amino acid profiles and digestibility, serving as key determinants (Atowa et al., 2021). It is noteworthy that the amino acid composition varied across different growth stages of the cicada. Table 3 provides insights into the nutritional quality of both essential and non-essential amino acids, obtained through comprehensive amino acid analysis. Our findings revealed that all three growth stages of the cicada contained ten essential and nine non-essential amino acids. Notably, the quantities of both essential and non-essential amino acids exhibited an upward trend as the cicada advanced in age. Among the growth stages, the highest content of essential amino acids was observed in the adult cicada ($p < 0.05$), whereas the highest content of non-essential amino acids was found in the late nymph ($p < 0.05$). Methionine, histidine, and arginine emerged as the most abundant essential amino acids across all growth stages. Furthermore, tyrosine, glutamic acid, and aspartic acid stood out as the most abundant non-essential amino acids, while asparagine was not detected at any growth stage. Interestingly, our study demonstrates that cicadas at all three growth stages serve as excellent sources of sulfur-containing amino acids, particularly methionine. These sulfur-containing amino acids hold significant importance in the body due to their antioxidant potential, aiding in the removal of reactive oxygen species and preventing cell damage caused by oxidative stress (Atowa et al., 2021; Fogang Mba et al., 2017; Wu et al., 2020). It is worth noting

Table 4
Fatty-acid composition in Thai cicada at different growth stages.

Parameter	Fatty acid content (g/100 g)		
	Nymph	Late nymph	Adult
C10:0 Capric acid	ND	0.01 ± 0.00^{a}	ND
C12:0 Lauric acid	ND	0.05 ± 0.00^{a}	0.04 ± 0.00^{b}
C14:0 Myristic acid	ND	0.26 ± 0.02^{a}	0.20 ± 0.01^{b}
C16:0 Palmitic acid	0.20 ± 0.01^{c}	3.12 ± 0.11^{a}	2.62 ± 0.09^{b}
C18:0 Stearic acid	4.03 ± 0.24^{a}	1.28 ± 0.05^{b}	1.06 ± 0.01^{c}
C20:0 Arachidic acid	7.16 ± 0.31^{a}	0.15 ± 0.01^{c}	0.19 ± 0.00^{b}
C21:0 Heneicosanoic acid	0.04 ± 0.00^{a}	ND	ND
C22:0 Behenic acid	10.50 ± 0.37^{a}	0.02 ± 0.00^{b}	ND
C16:1 n-7 Palmitoleic acid	ND	0.10 ± 0.00^{a}	0.07 ± 0.00^{b}
C18:1 n-9 *cis*-Oleic acid	2.10 ± 0.13^{c}	7.58 ± 0.07^{a}	6.82 ± 0.16^{b}
C20:1 *cis*-11-Eicosenoic acid	0.04 ± 0.00^{a}	0.01 ± 0.00^{c}	0.02 ± 0.00^{b}
C24:1 n-9 Nervonic acid	ND	0.06 ± 0.00^{b}	0.07 ± 0.00^{a}
C18:2 n-6 *cis*-Linoleic acid	6.90 ± 0.15^{a}	1.99 ± 0.03^{c}	4.03 ± 0.23^{b}
C22:6 n-3 Docosahexaenoic acid	ND	ND	ND
C20:4 n-6 Arachidonic acid	ND	ND	ND
C20:5 n-3 *cis*-5,8,11,14,17-Eicosapentaenoic acid	0.88 ± 0.03^{a}	0.26 ± 0.01^{b}	0.23 ± 0.01^{c}
SFA	21.93 ± 0.15^{a}	4.89 ± 0.25^{b}	4.09 ± 0.13^{c}
MUFA	2.14 ± 0.09^{c}	7.59 ± 0.31^{a}	6.84 ± 0.24^{b}
PUFA	7.78 ± 0.05^{a}	2.29 ± 0.06^{c}	4.31 ± 0.05^{b}
Total	31.85 ± 0.28^{a}	14.77 ± 0.04^{c}	15.24 ± 0.22^{b}
n-3	0.88 ± 0.01^{a}	0.26 ± 0.00^{b}	0.23 ± 0.00^{c}
n-6	6.90 ± 0.10^{a}	1.99 ± 0.09^{c}	4.03 ± 0.17^{b}
n-9	2.10 ± 0.12^{c}	7.64 ± 0.23^{a}	6.90 ± 0.13^{b}

Values are expressed as mean ± SD of triplicate measurements (n = 3). Means with different letters are significantly different at $p < 0.05$ within the same row in the parameter. ND: Not detected; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

that the amino acid contents of edible insects can vary significantly, ranging from 90 mg/g in *Rhynchophorus phoenicis* larvae (Fogang Mba et al., 2017) to 931 mg/g in *Zophobas morio* (Yi et al., 2013). In our study, the overall amino acid content in cicadas was found to be moderate among edible insects. The abundant amino acid content observed in these insect species highlights their potential role in the human diet, providing essential amino acids and serving as an economical protein source for meals (Atowa et al., 2021).

The EAAI (Essential Amino Acid Index) for cicadas at different growth stages, including nymphs, late nymphs, and adults, was calculated and presented in Table 3. The essential amino acid contents considered for EAAI calculation are lysine, methionine, phenylalanine, threonine, histidine, isoleucine, leucine, tryptophan, and valine, as established by Yi et al. (2013). Remarkably, the EAAI values exhibited a significant increase from 0.12 in nymphs to 0.36 in adults ($p < 0.05$). These values fall within the same range as mealworms (0.35) and crickets (0.45), but are lower than those of chicken (0.88), tuna (0.90), and beef (0.87), as reported by Lautenschläger, Neinhuis, Kikongo, Henle, and Förster (2017). Therefore, protein complementation may be

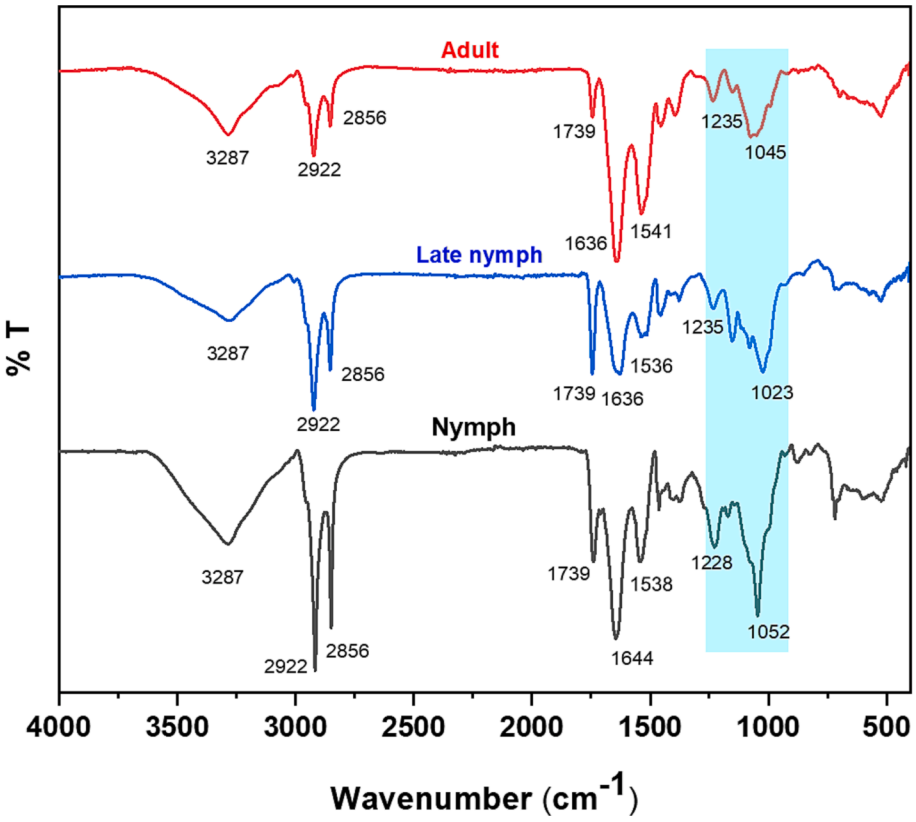

Fig. 1. Spectra of FT-IR in Thai cicada at different growth stages.

necessary for improving the quality of protein from the cicadas.

3.5. Fatty-acid composition

Fatty acids possess anti-inflammatory properties and exert an influence on prostaglandin biosynthesis, thereby playing a significant role in skeletal-muscular diseases. Moreover, they serve as valuable sources of energy and essential fatty acids in the human diet (Wu et al., 2020). The fatty acid composition of cicadas was analyzed using gas chromatography, and the results are summarized in Table 4. Notably, the fatty acid profiles exhibited variations corresponding to the different growth stages. It is worth mentioning that most insects possess the capacity to biosynthesize palmitic, stearic, and oleic acids, which aligns well with the findings reported by Paul et al. (2017). The total fatty acid content was found to be highest in nymphs, followed by adults and late nymphs ($p < 0.05$). The saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) contents in the cicadas at three growth stages were 4.09–21.93, 2.14–7.59, and 2.29–7.78 g/100 g, respectively. Among the analyzed nymphs, SFA and PUFA were the most abundant fatty acids, whereas MUFA was predominant in late nymphs. These findings are consistent with the observations made by Atowa et al. (2021), who reported MUFA contents ranging from approximately 3 to 8 g/100 g in *Zonocerus variegatus*, *Macrotermes bellicosus*, and *Cirina forda*. Additionally, the PUFA contents were found to be in the range of about 2.5–15 g/100 g in their study. Interestingly, eicosapentaenoic acid (EPA), a key fatty acid in fish lipids, was also detected in cicadas at levels ranging from 0.23 to 0.88 g/100 g. The nymph demonstrated a high concentration of behenic acid ($p < 0.05$), while *cis*-oleic acid was abundant in the late nymph and adult ($p < 0.05$). Oleic acid plays a beneficial role in insulin sensitivity and resistance, as highlighted by Atowa et al. (2021) and Wu et al. (2020). The nymph serves as a notable source of n-6 essential fatty acids, which mammals must obtain from their diet as they cannot synthesize it (Wu et al., 2020). Conversely, the late nymph and adult exhibit richness in n-9 fatty acids, which can lead to decreased production of proinflammatory cytokines, increased IL-10 production, reduced neutrophil migration and accumulation at the infection site, and improved bacterial clearance (Medeiros-de-Moraes et al., 2018).

3.6. FT-IR analysis

Fourier-transform infrared (FT-IR) spectroscopy analysis stands as an effective approach for elucidating the distinctive functional groups and linkage bonds present in polysaccharides (Tian et al., 2021). In Fig. 1, we can observe the FT-IR spectra within the range of 400 to 4000 cm^{-1}, representing the cicadas at various growth stages. Remarkably, the results demonstrated similar characteristic peaks across all samples. The broad band observed at 3287 cm^{-1} likely arises from the hydroxyl group stretches of polysaccharides (Tian et al., 2021), and this band (3287 cm^{-1}) was asymmetric stretching vibrations of polymetric hydroxyl groups (O—H) and H-bonded stretching, which are characteristics of polyphenolic compounds. Additionally, the stretching related to the phenolic C—O bond was detected at approximately 1235 cm^{-1}, associated with the C—O configuration found in a pyran ring, a typical feature of the flavonoid C-ring (Wongsa et al., 2022). Furthermore, the sharp adsorption peak at 2922 cm^{-1} and 2856 cm^{-1} corresponds to the symmetric stretching of aliphatic compounds containing $–CH_3$ and –CH groups (Kaya et al., 2016).

In regard to specific functional groups, the peak at 1739 cm^{-1} indicates the presence of ester fatty acid groups containing the C═O bond, while the Amide I peak at 1636 cm^{-1} originates from the C═O stretching of the protein-peptide backbone (Jakinala et al., 2021; Olademehin et al., 2020). The Amide II region, ranging from 1500 to 1580 cm^{-1}, is assigned to in-plane bending vibrations of the N—H bond, as well as stretching vibrations of C—N and C—C bonds (Olademehin et al., 2020). According to the explanation by Lozano et al. (2017), the spectral

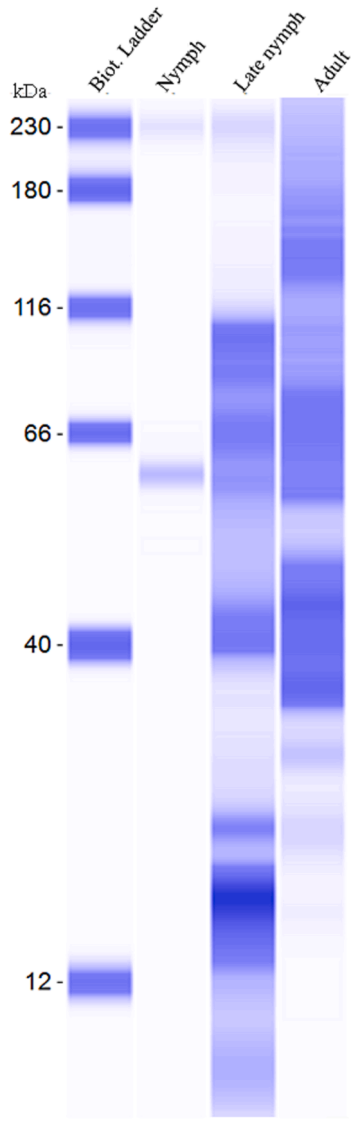

Fig. 2. Molecular weight distribution of proteins in Thai cicada at different growth stages.

peaks present at 1644, 1538 cm^{-1} (nymph), 1636, 1536 cm^{-1} (late nymph), and 1636, 1541 cm^{-1} (adult), as shown in Fig. 1, are characteristic of protein structures similar to those of other animals such as pork, lamb, chicken, beef, fighting bull, rabbit, hen, cow, and foal. Furthermore, the strong adsorption band at 1211 cm^{-1} confirms the existence of oligo- and polysaccharides through the symmetric stretching of C—O—P and P—O—P bonds (Jakinala et al., 2021; Kaya et al., 2016). The spectral peaks at 1200–900 cm^{-1} correspond to C—O—C stretching vibrations, thereby indicating the presence of polysaccharides, carbohydrates, and chitin (Kaya et al., 2016; Olademehin et al., 2020). Moreover, the band observed at 892 cm^{-1} relates to the stretching vibrations of the C═O bond within inorganic carbonates (Jakinala et al., 2021). Additionally, the nymph stage exhibited a higher adsorption intensity of functional groups compared to the late nymph and adult stages, indicating a greater abundance of functional groups within its structure. This finding aligns with the elevated levels of phenolic acids, flavonoids, tocopherols, γ-oryzanol, and fatty acids observed in the nymph stage.

3.7. Protein molecular weight

The molecular-weight distribution of proteins in the cicada extracts is shown in Fig. 2. As can be seen, the bands exhibited greater intensities in the late nymph and adult stages, indicating higher protein content in those samples. This observation aligns with the trend observed in the amino-acid composition of cicadas, as presented in Table 3. Specifically, it was discovered that approximately 70 % of the proteins had a molecular weight below 66 kDa in the late nymph and adult cicadas. Within the cicada supernatant fractions, a range of bands spanning from 12 to 92 kDa were observed, likely attributed to various proteins and enzymes. Examples of such proteins include melanization-inhibiting protein (43 kDa), β-glycosidase (59 kDa), trypsin-like proteinases (59 kDa), and melanization-engaging protein variants (85 kDa) (Yi et al., 2013). Interestingly, protein bands over 75 kDa were present in all extracts obtained from the defatted cicadas. However, the nymph and late nymph samples exhibited either no or very faint protein bands above 75 kDa after extraction. This finding is consistent with previous reports on *Tenebrio molitor* and *Allomyrina dichotoma*, which also demonstrated a lack of or faint protein bands above 75 kDa following extraction (Kim et al., 2020). Furthermore, the adult cicada extract exhibited diverse protein band patterns, showcasing a wide distribution in SDS-PAGE profiles. Therefore, based on our results and utilizing capillary-based western analyses, it can be inferred that the adult stage may possess enhanced functionality compared to the nymph and late nymph stages, as the protein bands in the adult extract displayed a more extensive dispersion.

4. Conclusion

In this study, the nutritional and bioactive components of the cicada at different growth stages, including nymph, late nymph, and adult, were investigated. As expected, the maturation of cicada significantly affects its nutritional and bioactive values. The nymphs seem to be the best source of α-, δ-, and γ-tocopherols, γ-oryzanol, phenolic acids, and flavonoids, and they possess a more appropriate ratio of n-3 to n-6 fatty acids and higher amount of PUFA, whereas the late nymphs and adults exhibit a higher protein content and a more balanced amino acid profile, making them a valuable protein source. The fatty acid analysis reveals the presence of beneficial fatty acids, especially EPA, which was found in all the samples, ranging from 230 mg/100 g in adults to 880 mg/100 g in nymphs. The FT-IR spectroscopy confirms the presence of polysaccharides and proteins and indicates a greater abundance of functional groups during the stage of nymph as well. Moreover, the protein profile analysis shows distinct band patterns, with the adult cicadas displaying a broader range. To our knowledge, this is the first time researchers have reported the nutritional quality of cicada at different growth stages. These findings indicate the potential of cicadas as a sustainable and nutritious food source with functional benefits that can be exploited by food industry for the enrichment and fortification of human food. Although cicadas can serve as a potential source of bioactive substances for food processing, it is imperative to conduct a thorough investigation of the microbiological aspects and potential allergens when considering their use in freeze dried form, to ensure safety and quality. Meanwhile, further research is needed to explore their sensory attributes, processing methods, and potential health impacts. Incorporating cicadas into our diet could contribute to food security and promote a more sustainable food system.

CRediT authorship contribution statement

Hua Li: Conceptualization, Writing – original draft, Writing – review & editing. **Theeraphan Chumroenphat:** Data curation, Formal analysis, Investigation. **Apichaya Bunyatratchata:** Data curation, Formal analysis, Investigation. **Parinya Boonarsa:** Data curation, Formal analysis, Investigation. **Colin Wrigley:** Writing – review & editing.

Sirithon Siriamornpun: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.101081.

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