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Characterisation of nutritional, physiochemical, and biological properties of furikake cricket seasoning powder

[1]*Somdee, T., [1]Yangyuen, S., [1]Srikongpan, P., [1]Nonsa-ard, R., [1]Thitisutthi, S., [2]Mungvongsa, A., [3]Chumroenphat, T. and [4]Somdee, T.

[1]*Faculty of Public Health, Mahasarakham University, Maha Sarakham 44150, Thailand*
[2]*Institute of Public Health, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand*
[3]*Aesthetic Sciences and Health Program, Faculty of Thai Traditional and Alternative Medicine, Ubon Ratchathani Rajabhat University, Ubon Ratchathani 34000, Thailand*
[4]*Faculty of Science, Khon Kaen University, Khon Kaen 40000, Thailand*

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Abstract

Cricket (*Gryllus bimaculatus* De Geer) is an abundant source of protein and amino acids in addition to its biological properties. In the present work, crickets were processed into four formulations of furikake cricket seasoning powder (FCSP) containing different amounts (54 - 60%) of cricket powder. The nutritional value, amino acid profile, physiochemical properties, and biological properties, including antioxidant compounds, activity, and cytotoxicity were measured, and sensory evaluation was performed. FCSP showed high protein (37.13 - 50.87%), fat (20.16 - 22.96%), and leucine and valine contents. However, histamine content was lower than 4 µg/g in all formulations. The antioxidant compounds and activity of the FCSP were enhanced with increasing amount of cricket powder. Moreover, the extract induced cell death in HepG2. Sensory analysis showed that the cricket powder enhanced the taste and overall acceptability. Therefore, FCSP could be a novel and alternative seasoning product that can enhance nutritional value, and potentially promote consumer well-being. However, further mechanistic and clinical studies are needed to substantiate its health effects.

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Introduction

Insects are consumed in many countries as a sustainable and nutritious alternative food source. Moreover, the health effects of their consumption have attracted global attention (Cunha *et al*., 2023). As reported by the Food and Agriculture Organization (FAO) of the United Nations (UN), edible insects are a source of high-quality protein and essential fatty acids (FAO, 2013; Loypimai *et al*., 2024). They are also rich in essential amino acids required for human health. Variation in amino acid profiles has been observed among different insect species (Ojha *et al*., 2021). At present, edible insects are promoted as a food of the future because they contain high-quality protein and micronutrients associated with economic and environmental benefits (Lange and Nakamura, 2021).

Among edible insects, cricket (*Gryllus bimaculatus* De Geer), with a protein content of 58.32% and a fat content of 11.88%, is one of the most interesting options, partly due to its low cost (Ghosh *et al*., 2017). A previous study has shown that crickets are an attractive but challenging option for functional food; they have desirable nutritional values, a pleasant nutty umami flavour, and good functional properties (Udomsil *et al*., 2019). Thus, it is important to determine the functional properties, including antimicrobial, antioxidant, and anticancer activities, and understand their use as functional ingredients in the food industry (Chakrabarti *et al*., 2018; Malm and Liceaga, 2021; Lim and Byun, 2021).

To develop uses for insects in food products and promote their acceptance, factors of concern for novel and alternative foods must be considered, such as modifying the appearance of the food to enhance its acceptance. Cricket products in the form of snacks and powders have been formulated to prevent recognition of the crickets, and increase their food industry market potential (Kemsawasd *et al*., 2022). However, large numbers of crickets have a distinct

*Corresponding author.
Email: thidarat@msu.ac.th

odour, which hinders market potential, and must be improved. Therefore, developing food products from cricket requires achieving public acceptance, reducing the distinct odour, and maintaining nutritional value and biological activity.

The global seasoning and spices market size was valued at USD 37.26 billion in 2022, and an increasing demand for seasoning powder is expected. This demand is expected to grow at a compound annual growth rate of 5.6% from 2023 to 2030 (statistics from market analysis report). The demand for seasoning without MSG, such as furikake (a rice topping), presents an opportunity to develop and promote healthier alternatives. Prior work with novel products containing cricket protein in China and Thailand has revealed that Chinese consumers favour product concepts such as nutritional supplements and culinary seasonings. In Thailand, consumer acceptance is higher when crickets are used in processed food products, with cricket powder being the most commonly accepted in desserts and staple dishes (Wannasupchue and Wongthahan, 2024).

Several cricket-based food products are available on the market, but they are limited to furikake seasoning powder with herbs added that reduce the distinct odour of cricket. However, cricket is a good source of umami taste. Therefore, the objectives of the present work were fourfold: (1) to develop furikake using cricket; (2) to examine the nutritional value and histamine content, amino acid profile, and physiochemical properties of the developed products; (3) to evaluate its biological properties, including antioxidant compounds, activity, and cytotoxicity to HepG2; and (4) to analyse the level of acceptance of FCSP through sensory evaluation.

Materials and methods

Materials

Fresh crickets, around seven weeks old, were obtained from a commercially operated cricket farm situated in Maha Sarakham, Thailand. Additional ingredients were obtained from a nearby marketplace also in Maha Sarakham. The crickets underwent cleaning, and drying using a hot air oven set at 80°C until completely dehydrated to reach $a_w < 0.6$. The cricket powder was sieved through a 0.5-mm mesh sieve, and spread evenly on a stainless-steel plate. After that, the FCSP formulations were prepared, and their ingredients are given in Table 1. The fine cricket powder was added at 54, 56, 58, and 60%. All chemicals used to determine the nutritional value, amino acid profile, and biological properties were of analytical reagent grade.

Table 1. Formulation of FCSP.

Ingredient	Amount (%)			
	A	B	C	D
Cricket powder	60	58	56	54
White sesame	10	10	10	10
Black sesame	8	8	8	8
Soy sauce	8	8	8	8
Sugar	8	8	8	8
Lemon grass	2	2	2	2
Shiitake mushroom	4	6	8	10

Determination of nutritional value

The moisture content, ash, crude fat, crude protein, and total carbohydrates of the FCSPs obtained from the different formulations were assessed based on the protocols outlined by the AOAC official methods (AOAC, 2016). Briefly, the moisture content was determined using an electric oven at 105°C for 6 h, and drying the samples to a constant weight. Analytical measurements were conducted in triplicate for each sample. The ash content was examined using a muffle furnace at 600°C for 12 h. The fat content was examined using the Soxhlet method for 2 h with petroleum ether. The Kjeldahl procedure was employed to quantify the protein content, which consisted of three-step acid digestion, distillation, and titration, and calculated by multiplying the nitrogen percentage of the digestate by 6.25. To determine total carbohydrates, the proportions of moisture, protein, lipid, and ash were summed and subtracted from 100%.

Determination of histamine content

The ELISA technique was employed to measure the histamine concentration in the samples using a histamine/ ELISA kit (Kikkoman Biochemifa Company, Japan).

Determination of amino acid profile

Amino acid profiles were assessed following the method of Chumroenphat *et al*. (2019). Briefly, 2 g of sample of each FCSP formulation and a mixture of 0.5 mL of 0.05 M aqueous HCl and ethanol (1:1, v/v) was prepared and agitated using a vortex mixer for 5 min, and immediately centrifuged at 12,000 *g* at 4°C for 15 min. The samples were analysed by LC-

MS/MS using Shimadzu's LC-MS-8030 triple quadrupole mass spectrometer (ESI mode) and LC-20AC series high-performance liquid chromatography system. The mobile phase consisted of a mixture of (A) 0.1% formic acid in water, and (B) the solution was diluted with methanol (1:1) at a flow rate of 0.2 mL/min. The auto-sampler temperature was 4°C, and the injection volume was 2 µL. The mass spectrometry analysis utilised MS/MS multiple reaction monitoring (MRM) mode, with instrumental settings including a 4.5 kV capillary voltage (positive ESI), 1.72 kV cone voltage, and 400°C ion source temperature. Amino acids were identified by their characteristic *m/z* signals and retention time alignment with standard samples.

Determination of physicochemical properties

All the analyses of the physiochemical properties were conducted in triplicate, including water activity (a_w), colour, and calorie values. The a_w was determined using a water activity meter (LANDTEK WA-60A, Guangzhou, China). Colour measurements for L*, a*, and b* values were obtained using an NH300 handheld colorimeter (3NH, Shenzhen, China). The calorie values were measured using a bomb calorimeter (IKA calorimeter C2000 Basic, IKA-Werke, Germany).

Determination of biological properties

Preparation of sample extracts

FCSP samples weighing 0.5 g were extracted by soaking in 10 mL of ethanol overnight at room temperature. The mixture was filtered with Whatman No. 42 filter paper, followed by washing the residue with ethanol. The filtrate was then evaporated in a 50°C water bath to a final volume of 1 mL for use in biological property assessments (Somdee *et al*., 2023).

Antioxidant compounds

Colorimetric methods were used to quantify the total phenolic content (TPC) and total flavonoid content (TFC). TPC determination was conducted following the Folin-Ciocalteu procedure (Yawadio *et al*., 2008). An aliquot of 0.5 mL diluted sample was introduced to 2.5 mL of Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate, then incubated at room temperature for 30 min. Readings of absorbance at 725 nm were recorded, employing gallic acid as the calibration standard; TPC values were expressed as mg gallic acid equivalents.

The TFC was determined following Abu-Bakar *et al*. (2009). An aliquot of 500 µL sample was combined with 2.25 mL of distilled water and 0.15 mL of 5% $NaNO_2$ solution, followed by a 6-min incubation. Next, 0.3 mL of 10% $AlCl_3$ was added and incubated for 5 min. Lastly, 1.0 mL of 1 M NaOH was added, with TFC reported as mg catechin equivalents.

Antioxidant activity

i) 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH antioxidant test was conducted following a modified version of the procedure of Brand-Williams *et al*. (1995). To assess the antioxidant activity, 0.2 mL of sample was reacted with 2.0 mL of 0.20 mM DPPH in ethanol. The mixture was incubated for 30 min in the dark at room temperature, the absorbance was then measured at 517 nm, and Trolox was used as a standard. Scavenging activity against DPPH radicals was calculated as follows: % inhibition = 100 × [1 − (AE / AD)]; where, AE = absorbance in the presence of the sample extract, and AD = absorbance of the DPPH control solution.

ii) Ferric-reducing antioxidant potential (FRAP) activity

FRAP assay was conducted following the method described by Benzie and Strain (1999). Briefly, the FRAP reagent was freshly prepared by mixing 1 mL of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, 1 mL of 300 mM acetate buffer (pH 3.6), and 1 mL of 20 mM $FeCl_3 \cdot 6H_2O$. Then, 100 µL of the sample was added to 1 mL of the FRAP reagent, and incubated in the dark for 30 min. The absorbance was measured at 593 nm, and the FRAP value was calculated using a standard curve of $FeSO_4 \cdot 7H_2O$.

Cytotoxicity activity

The cytotoxic effects of FCSP extracts on HepG2 cell viability were evaluated using the sulforhodamine B (SRB) assay, as described by Buranrat *et al*. (2017). HepG2 cells were treated with various concentrations of the extract (0 – 1,000 µg/mL) for 24 or 48 h. Following treatment, cells were fixed with 10% trichloroacetic acid, stained with 0.4% SRB, washed with 1% acetic acid, and solubilised in 10 mM Tris base. Doxorubicin was employed as a positive control in the SRB assay. The

absorbance was measured at 540 nm using a spectrophotometer. The cell inhibition was calculated as follows: % cell inhibition = 100 − [(As − Ab) / (Ac − Ab)] × 100; where, As = absorbance of the sample, Ab = absorbance of the blank, and Ac = absorbance of the control (Patel *et al*., 2009).

Sensory evaluation

The sensory analysis was conducted by an untrained panel at Maha Sarakham University selected from the staff and students (50 individuals between 18 and 45 years old). A panel of 50 untrained participants evaluated the FCSP using a 9-point hedonic scale. The study was approved by the Ethics Committee of Maha Sarakham University (approval no.: ECMSU196-168/2023).

Statistical analysis

All experiments were conducted in three technical replicates. The data were recorded as mean ± standard deviation, and analysed using SPSS (version 11.5 for Windows 98, SPSS Inc.). One-way ANOVA followed by Tukey's *post hoc* test was used to determine significant differences, with $p < 0.05$ considered statistically significant.

Results and discussion

Nutritional properties of FCSP

The proximate composition of FCSP is shown as percentages in Table 2. The powder containing only cricket contained 7.40% moisture, 52.07% protein, 12.25% fat, 2.52% ash, and 25.76% total carbohydrate per 100 g sample. Cricket was added to the seasoning powders to enhance their nutritional value. The moisture content in the FCSPs was around 7.00 - 7.76%, while the ash content ranged from 5.17 - 5.85%. Moreover, the fat content of FCSP ranged between 20.16 and 22.96%, indicating that the powders were rich in minerals. Interestingly, the protein content (37.13 - 50.87%) significantly increased ($p = 0.032$) with cricket content. Studies by Ghosh *et al*. (2017) have shown that cricket is an alternative high-protein source with 58.32% dry weight, which is similar to the amount of protein in chicken eggs and meat (Mlcek *et al*., 2014; Kim *et al*., 2019). Therefore, FCSP could be considered a high-protein food source, according to WHO/FAO (WHO, 2007).

The reported fat content of FCSP was between 20.16 and 22.96%, which increased with added cricket powder. This agreed with a previous study that revealed that the fat content of food products will increase with the incorporation of cricket powder (Smarzyński *et al*., 2019). Generally, crickets are known as a source of fat because fat is the second largest component of cricket, with a fat content of about 25.14% (Mlcek *et al*., 2014; Kim *et al*., 2019). Additionally, crickets are a source of palmitic, oleic, and linoleic acids (Kim *et al.*, 2016). The carbohydrate content of FCSPs produced with cricket powder ranged from 13.51 - 28.78%; there was a significant ($p = 0.030$) reduction with cricket powder content. The reduced carbohydrate content might have been due to the increased ash, fat, and protein contents. All FCSPs exhibited histamine levels lower than 4 µg/g, making the FCSP a safe food product with a histamine limit not exceeding 100 mg/kg (Rauscher-Gabernig *et al.*, 2009). Therefore, FCSP could be an alternative protein and fat source with natural products, as well as improving the nutritional status of consumers.

Table 2. Nutritional properties of FCSP.

Nutrition composition (%)	FCSP			
	A	B	C	D
Moisture	7.00 ± 0.48^{b}	7.59 ± 0.78^{a}	8.74 ± 1.07^{a}	7.76 ± 1.37^{a}
Ash	5.66 ± 0.09^{a}	5.79 ± 0.06^{a}	5.85 ± 0.08^{a}	5.17 ± 0.34^{b}
Fat	22.96 ± 0.93^{a}	20.75 ± 0.24^{b}	20.86 ± 0.90^{b}	20.16 ± 0.53^{b}
Protein	50.87 ± 0.81^{a}	48.44 ± 0.20^{b}	40.01 ± 0.51^{c}	37.13 ± 1.34^{d}
Crude fibre	2.91 ± 0.10^{d}	3.61 ± 0.16^{c}	4.35 ± 0.13^{b}	5.64 ± 0.21^{a}
Carbohydrate	10.60 ± 0.07^{d}	14.82 ± 0.20^{c}	20.19 ± 0.03^{b}	22.60 ± 0.13^{a}
Histamine (µg/g)	< 4	< 4	< 4	< 4

Values are mean ± SD of triplicate measurements ($n = 3$). Means with different lowercase superscripts in each row are significantly different ($p < 0.05$).

Amino acid evaluation

The total amino acid composition of FCSP is shown in Table 3; the concentration of leucine was the highest (2505.73 to 3191.45 µg/g) in all the samples, while tryptophan was the lowest (15.85 to 23.31 µg/g) among the essential amino acids. The pure cricket powder contained the highest amounts of leucine and valine, 4218.02 and 2784.18 µg/g, respectively. Defoliart (1992) has shown that isoleucine, leucine, and valine were the most abundant amino acids in house crickets. The amino acid profile showed that cysteine and methionine abundance could be low in insects, while lysine and threonine could be high. Additionally, FCSPs are a source of protein, meeting the WHO (1973) requirements of 40% essential amino acids, and all FCSPs are more than 70% essential amino acids.

Of the non-essential amino acids, glycine showed the highest concentrations (387.92 - 465.46 µg/g), followed by glutamic acid (151.30 - 384.66 µg/g). Kohler *et al*. (2019) have reported that the house cricket had the highest concentration of glutamic acid. Furthermore, the amounts of umami amino acids, including aspartic and glutamic acids, determined from the total amino acid content ranged from 2.80 to 4. 20%. The recommended amino acids for athletes include branched-chain amino acids (leucine and valine), which are critical for skeletal muscles (Mann *et al*., 2021), and are in FCSP. The limiting amino acid of FCSP was tryptophan, which was found in house crickets (Kohler *et al*., 2019).

Table 3. Amino acid composition of FCSP.

Amino acid	Content (µg/g)			
	A	B	C	D
		Essential		
Arginine	492.70 ± 10.52^{a}	485.58 ± 7.21^{a}	355.79 ± 10.32^{b}	308.42 ± 12.83^{c}
Histidine	507.76 ± 17.91^{a}	500.57 ± 9.19^{a}	405.98 ± 11.41^{b}	351.20 ± 5.83^{c}
Isoleucine	656.9 ± 18.01^{a}	655.79 ± 9.11^{a}	460.49 ± 4.26^{b}	400.60 ± 33.16^{c}
Leucine	3491.45 ± 38.82^{a}	3109.03 ± 16.85^{b}	3044.98 ± 22.21^{c}	2505.73 ± 13.46^{d}
Lysine	428.85 ± 9.59^{a}	410.54 ± 2.54^{a}	336.67 ± 2.48^{b}	167.51 ± 2.76^{c}
Methionine	288.68 ± 5.65^{a}	270.07 ± 23.27^{a}	199.47 ± 5.10^{b}	134.66 ± 9.35^{c}
Phenylalanine	393.43 ± 4.90^{a}	404.96 ± 6.52^{a}	321.11 ± 13.27^{b}	415.46 ± 9.93^{a}
Threonine	42.82 ± 2.16^{b}	47.85 ± 0.77^{a}	39.81 ± 3.71^{c}	50.51 ± 3.74^{a}
Tryptophan	18.53 ± 0.77^{b}	18.96 ± 2.11^{b}	15.85 ± 0.63^{c}	23.31 ± 0.67^{a}
Valine	2146.84 ± 30.52^{a}	1923.71 ± 9.71^{b}	1631.46 ± 23.19^{c}	1120.74 ± 3.56^{d}
Sum	8467.96 ± 138.85^{a}	7827.06 ± 87.28^{b}	6811.61 ± 96.58^{c}	5478.14 ± 95.29^{d}
		Non-essential		
Alanine	342.69 ± 20.55^{a}	262.85 ± 4.16^{b}	202.05 ± 9.70^{c}	72.70 ± 13.82^{d}
Asparagine	40.74 ± 0.52^{ns}	39.45 ± 0.68^{ns}	40.42 ± 0.79^{ns}	41.88 ± 0.27^{ns}
Aspartic acid	35.88 ± 2.79^{ns}	37.65 ± 1.80^{ns}	35.00 ± 0.66^{ns}	35.35 ± 0.99^{ns}
Cysteine	ND	ND	ND	ND
Glutamine	290.57 ± 2.95^{c}	303.65 ± 2.34^{b}	303.76 ± 17.55^{b}	515.79 ± 7.65^{a}
Glutamic acid	384.66 ± 8.29^{a}	371.24 ± 3.29^{a}	310.20 ± 4.04^{b}	151.30 ± 0.66^{c}
Glycine	465.46 ± 14.83^{a}	448.52 ± 3.91^{ab}	415.74 ± 2.02^{b}	387.92 ± 3.96^{c}
Proline	204.54 ± 13.00^{a}	197.73 ± 3.02^{a}	147.79 ± 2.22^{b}	95.39 ± 3.77^{c}
Serine	140.34 ± 1.99^{a}	139.01 ± 9.08^{ab}	130.92 ± 6.11^{b}	130.4 ± 1.69^{b}
Tyrosine	227.80 ± 10.77^{b}	257.92 ± 10.89^{a}	174.91 ± 4.23^{b}	114.62 ± 8.30^{c}
Sum	2132.64 ± 95.69^{a}	2058.02 ± 75.17^{a}	1760.79 ± 47.32^{b}	1545.35 ± 41.11^{c}

Values are mean ± SD of triplicate measurements (n = 3). Means with different lowercase superscripts in each row are significantly different ($p < 0.05$).

Physiochemical properties

The physical properties of the FCSPs are shown in Table 4. Crickets are the key raw material. In crickets, the a_w and colour determine the influence of the cricket powder. Comparing the a_w of each FCSP formulation showed no significant differences between them. Furthermore, the a_w of the FCSPs were safe for consumption according to the quality control guidelines in USFDA (2018) regulations, which state that a_w should be below 0.60, because an a_w between 0.60 and 0.85 is associated with the growth of pathogenic and spoilage microorganisms. The colour of the formulation changed with the amount of cricket powder, but the lightness (L* value) did not differ significantly. The FCSP exhibited significantly decreased red (a* value) and yellow (b* value) shades with decreased cricket powder content. The change possibly resulted from the oxidation of lipid components in the raw material (Cho *et al*., 2018). It was expected that the physical properties (a_w and colour) of the FCSPs would vary depending on the amount of added cricket powder.

Additionally, the calorie value differed significantly ($p < 0.05$) for all FCSPs (Table 4), for which Formula A (60% cricket powder) had the highest value. The present work used a bomb calorimeter to analyse the calorie value of the FCSPs, which yields a caloric density that includes the calories from digestible and indigestible components, such as fibre (Grobe, 2017). Thus, the high fibre content led to increased calorie values obtained from the shiitake mushroom content.

Biological properties

Antioxidant compounds

The changes in the antioxidant compound contents (TPC and TFC) in the FCSPs are shown in Table 5. The TPC decreased from 61.10 ± 0.21 to 78.02 ± 1.23 mg gallic acid equivalents/g dry weight, and there were significant differences for each FCSP formulation ($p = 0.024$) with decreased amounts of cricket powder, with Formula A having the highest value. Moreover, the TFC was evaluated as a proxy for biological compounds in FCSP. TFC trended with TPC, but bioactive compounds, including TPC and TFC, depended on the cricket powder content.

However, TPC was higher than TFC in all formulations. The TPC content in the present work

Table 4. Physical properties of FCSP.

Physical properties	FCSP			
	A	B	C	D
a_w	0.59 ± 0.02^{ns}	0.60 ± 0.00^{ns}	0.59 ± 0.00^{ns}	0.60 ± 0.00^{ns}
Colour				
L*	50.46 ± 0.27^{ns}	50.46 ± 1.12^{ns}	50.40 ± 0.93^{ns}	50.05 ± 1.02^{ns}
a*	7.02 ± 0.04^{d}	7.45 ± 0.07^{c}	7.48 ± 0.12^{b}	7.78 ± 0.07^{a}
b*	12.24 ± 0.42^{d}	13.01 ± 0.32^{c}	13.84 ± 0.16^{b}	14.27 ± 0.11^{a}
Calories (kcal/100 g)	235.76 ± 1.15^{a}	228.98 ± 0.23^{b}	225.55 ± 1.79^{b}	220.55 ± 1.02^{c}

Values are mean ± SD of triplicate measurements ($n = 3$). Means with different lowercase superscripts in each row are significantly different ($p < 0.05$). ^{ns}non-significant.

Table 5. Antioxidant activity and compounds of FCSP.

Sample	Antioxidant activity		Antioxidant compound	
	DPPH (% scavenging activity)	FRAP (mmol $FeSO_4$/g dry weight)	TPC (mg/g dry weight)	TFC (mg/g dry weight)
A	78.02 ± 1.23^{a}	68.69 ± 0.73^{a}	575.03 ± 2.80^{a}	439.15 ± 1.09^{a}
B	71.39 ± 0.84^{b}	61.95 ± 1.05^{b}	530.12 ± 1.13^{b}	391.15 ± 2.51^{b}
C	66.71 ± 1.23^{c}	56.12 ± 0.56^{c}	471.82 ± 1.01^{c}	353.78 ± 2.07^{c}
D	61.10 ± 0.21^{d}	50.74 ± 0.81^{d}	432.27 ± 0.72^{d}	337.12 ± 2.33^{d}

Values are mean ± SD of triplicate measurements ($n = 3$). Means with different lowercase superscripts in each column are significantly different ($p < 0.05$).

was higher than that reported by previous studies. Previous studies have shown that the TPC content in oven-dried (140°C) house cricket (*Acheta domesticus* L.) powder was 125 mg gallic acid equivalents/g dry weight, and when not thermally treated, 148 mg gallic acid equivalents/g dry weight (Loypimai *et al*., 2024). Additional thermal processing noticeably influenced the availability of phenolic and flavonoid compounds compared to our procedure for cricket powder with oven drying at 80°C. Interestingly, cricket provides most phenolic compounds through enzymatic alterations of the shikimic acid pathway produced from amino acids with l-tyrosine (Loypimai *et al*., 2024). Flavonoids are phenolic compounds with biological activity, such as anti-inflammatory, antioxidant, and cytotoxicity properties in humans (Cheseto *et al*., 2020) and FCSP potentially had these properties.

Antioxidant activity

Antioxidant reactions with free radicals can be due to several reaction mechanisms (Santos-Sánchez *et al*., 2019), and our results were evaluated using two different methods, DPPH and FRAP assays (Table 5). All of the FCSPs showed high antioxidant activity (> 50%). This scavenging activity with inhibition increased significantly ($p < 0.05$). Meanwhile, the antioxidant activity values measured by the FRAP method are indicated as the Fe^{3+} reduction capacity equivalent to that of 1 mmol $FeSO_4$. A similar trend was found in the DPPH assays, where the highest activity was recorded in the FCSP formulation (A) containing the most cricket powder (68.69 mmol $FeSO_4$/g dry weight), and showed that the protein and amino acid of cricket powder may be factors on the antioxidant activity of the FCSP. Previous research has reported that antioxidant activity in cricket protein passed bioactive peptides composed of 2 to 20 amino acids, and these peptides have been shown to be able to modulate antioxidant activity (Chakrabarti *et al.*, 2018). These bioactive peptides can be manufactured by food processing, such as hot water and autoclaving (Jeong *et al*., 2021). The present work showed that FCSPs containing cricket powder could be a good alternative source of antioxidant power, as well as an alternative protein source.

Cytotoxicity

Cytotoxicity tests were performed on HepG2 cells, and cell viability was determined using the SRB assay. Figure 1 shows the viability of HepG2 cells when treated with FCSP for 24 and 48 h. We found that cells were viable for all FCSP samples with a dose-dependent manner. The HepG2 cell viability was significantly reduced by FCSP when tested at 100 – 1,000 µg/mL, except for the control sample. These results indicated that FCSP could induce a cytotoxicity effect that decreased the viability of the cells. Furthermore, these results supported findings related to the cytotoxic activity of cricket water extract, which inhibited H460 (cell lung carcinoma cell lines) proliferation through induced apoptosis of lung cancer cells possibly *via* caspase, Bcl-2 (Lim and Byun, 2021), and Summart *et al*. (2025) revealed that cricket-derived peptides could be effective anticancer agents *via* activating the intrinsic apoptotic pathway in cancer cells, which the intrinsic pathway was identified as the mechanism underlying apoptosis triggered by peptides P1 - P3 of cricket-derived peptides. In parallel, FCSP may elevate intracellular reactive oxygen species (ROS), which can amplify mitochondrial damage. Another research indicated that peptides with glutamic and aspartic acid residues have been shown to possess anti-proliferative potential against tumour cells (Yamaguchi *et al*., 2016). Thus, FCSP may have cytotoxic effects on cancer cell with selective binding to negatively charged membranes, leading to mitochondrial dysfunction, ROS generation, and activation of the intrinsic apoptotic pathway.

Sensory evaluation

Table 6 displays the mean sensory scores of FCSP by untrained consumers (50 individuals). The results showed that Formula A scored significantly higher than the other samples for appearance and taste ($p < 0.05$), which could be attributed to the presence of cricket powder. The result for the overall acceptability characteristics with increasing cricket powder content was similar. This result disagreed with the work of Suga *et al*. (2023), who found rice crackers with 10 - 30% cricket powder scored 20% with the highest overall sensory analysis result. However, the mean scores of flavour and flavour characteristics were not significantly different ($p > 0.05$). In the present work, we added a large amount of cricket powder (54 - 60%), which may have a distinct odour. Because the flavour scores were not significantly different, the lemongrass may have reduced the distinct odour of cricket. These

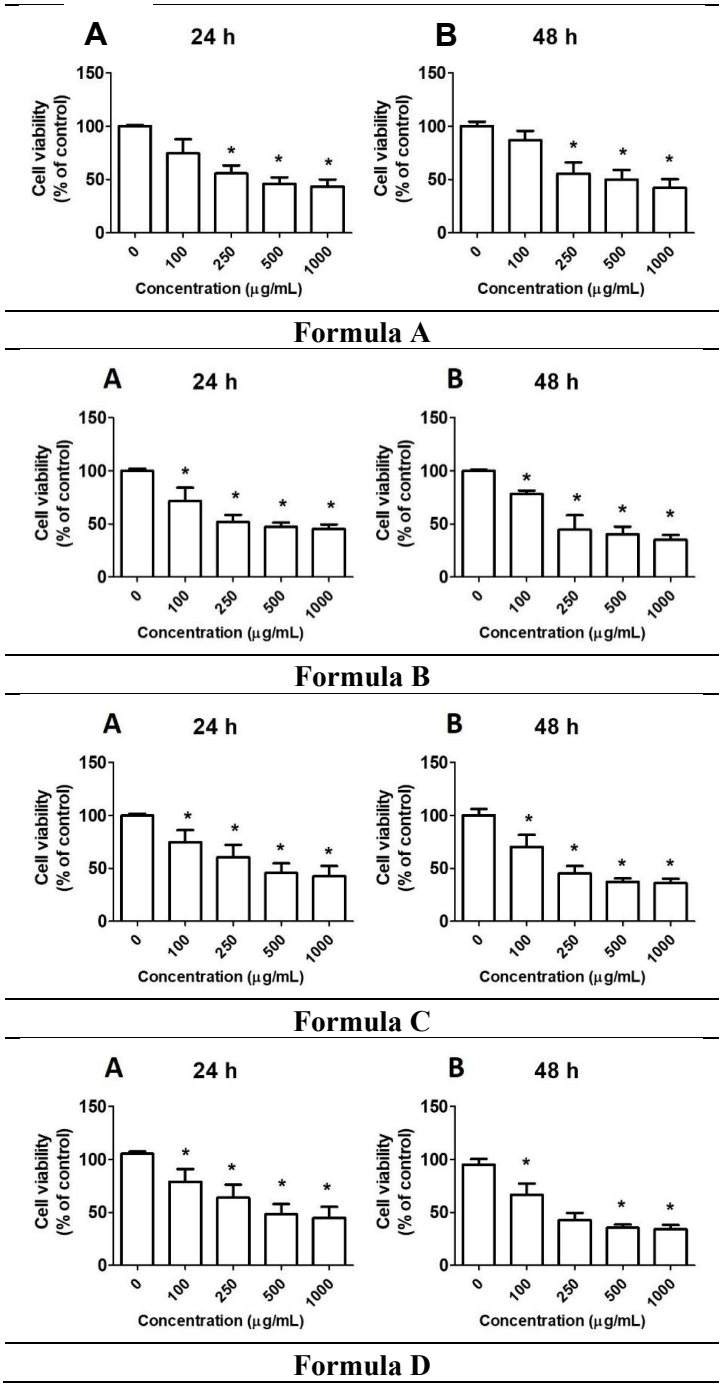

Figure 1. Cytotoxicity effects of FCSP at 24 and 48 h on HepG2 cancer cells. (*): $p < 0.05$ when compared with untreated control group.

Table 6. Sensory evaluation of FCSP.

Sensory test	FCSP			
	A	B	C	D
Appearance	6.43 ± 0.61^{a}	6.06 ± 0.15^{b}	5.93 ± 0.35^{c}	5.43 ± 1.03^{d}
Colour	6.03 ± 1.47^{ns}	6.13 ± 1.40^{ns}	6.20 ± 1.32^{ns}	6.26 ± 1.28^{ns}
Flavour	5.32 ± 0.69^{ns}	5.18 ± 0.65^{ns}	5.21 ± 1.01^{ns}	5.16 ± 0.94^{ns}
Taste	6.03 ± 1.56^{a}	5.90 ± 1.64^{b}	5.70 ± 1.62^{c}	5.36 ± 1.73^{c}
Overall acceptability	6.33 ± 1.58^{a}	6.20 ± 1.56^{b}	6.16 ± 1.55^{c}	5.76 ± 1.45^{d}

Values are mean ± SD of triplicate measurements ($n = 3$). Means with different lowercase superscripts in each row are significantly different ($p < 0.05$). ^{ns}non-significant.

characteristics showed the acceptability of FCSP, especially Formula A, which contained the most cricket powder, 60%.

Conclusion

Our results revealed that seasoning powder with cricket could be a potential alternative insect-based food protein source. We analysed the proximate composition, physicochemical, antioxidant activity, cytotoxicity, and sensory characteristics. FCSP is a high-protein food source, according to WHO/FAO, and has considerable nutritional value. It is a source of branched-chain amino acids, including leucine and valine, the primary amino acids needed for skeletal muscle. In the present work, FCSP expressed biological activity, especially cytotoxicity, with HepG2 cells. Nevertheless, the specific molecular mechanism needs to be further studied, and the bioactive components present in the cricket need to be identified. Knowledge gained from the present work could support the exploration of valuable uses of insect resources; moreover, this may be a primary study for further research into using different insects as new alternative foods. The novelty of the present work was in the development of furikake seasoning powder with a remarkably high inclusion level of cricket powder (54 - 60%), which exceeded the levels commonly reported in previous studies (typically 10 - 30%).

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