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RESEARCH ARTICLE
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Production of Protein Hydrolysates from Cricket using Bromelain and Its Application in Oyster Sauce Imitation Products

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ABSTRACT

The important variables affecting protein yield in the hydrolysate process are enzyme concentration and digestion duration. Optimal conditions were found to be a bromelain concentration of 0.75% and a digestion time of 6 hours, producing cricket protein hydrolysate containing 22.96% protein. Using cricket protein hydrolysate as the main ingredient in seasoning sauces offers an alternative to meet consumer demand for healthy seasoning products. Cricket sauces were formulated with varying amounts of cricket protein hydrolysate, salt, and low-sodium soy sauce (59.2, 1.7, and 4.2% respectively) and evaluated for their physical properties and sensory acceptance. The sauce made from cricket protein hydrolysate exhibited a high protein content (4.55%) compared to commercial oyster sauce, along with low fat (0.49%) and low sodium levels (165.9mg or 2.81%), meeting the physicochemical properties of the Thai Industrial Standard for oyster sauce (TIS 1317-2538). The application of 59.2% cricket protein hydrolysate in the seasoning sauce to mimic oyster sauce did not adversely affect taste, flavor, or overall acceptance. Therefore, cricket protein hydrolysis effectively increases protein content while reducing sodium levels in traditional oyster sauce formulations.

Keywords: Cricket sauce, Digestion time, Sensory acceptance, Low sodium.

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INTRODUCTION

The world population is currently estimated at 7.6 billion and is expected to increase to 9.8 billion by 2050 and 11.2 billion by 2100 (Adegboye et al., 2021; Tamburini et al., 2025). Consequently, food insecurity, particularly the lack of access to protein-rich diets, has become a critical global issue. Proteins can be categorized according to their sources: animal-based, such as meat, fish, milk, and eggs; and plant-based, including cereals, legumes, oilseeds, and microbiologically synthesized proteins. However, animal protein remains the most expensive source (Dolganyuk et al., 2023). Nowadays, protein deficiency is one of the most significant nutritional problems worldwide. In response to environmental changes and increasing demand, there is an urgent need to find alternative protein sources to replace conventional animal proteins. For this reason, the Food and Agriculture Organization of the United Nations (FAO) recommends the consumption of edible insects due to their high nutritional value and environmentally sustainable farming practices. Edible insects offer a promising new protein source for the growing global population. Insects are widely consumed as food or ingredients across Asia, Latin America, and Africa (Raheem et al., 2019; Magara et al., 2021). Currently, over 1,900 insect species are consumed by people in 128 countries worldwide. Asia is home to the greatest diversity of edible insects, followed by North America, Africa, South America, and Oceania. Mexico hosts more edible insect species than any other country, followed by Thailand, India, the Democratic Republic of Congo, China, Brazil, Japan, and Cameroon (Omuse et al., 2024; Sengendo et al., 2025). Common edible insects include crickets, moth caterpillars, ants, grasshoppers, and flies, among others (Van Huis, 2019; Peshuk et al., 2022; Papastavropoulou et al., 2023).

Crickets are among the edible insects recognized for their high protein content. In Thailand, the Thongdam and Sading species are the two most commonly consumed crickets. Thongdam Cricket (*Gryllus bimaculatus*) and Sading Cricket (*Acheta domesticus*), also known as the house cricket, both exhibit high protein levels ranging from 60% to 70% by dry weight, along with fat content between 10%

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and 23% (Udomsil et al., 2019). Cricket powder (*Acheta domesticus*) contains 42.0-45.8% protein and 23.6-29.1% fat and is rich in essential minerals, including Ca, mg, and Fe, as well as Cu, Mn, and Zn, which are particularly abundant ranging from 2.33-4.51mg, 4.1-12.5mg, and 12.8-21.8mg per 100g of dry matter, respectively (Montowska et al., 2019). This is consistent with findings by Chooklin et al. (2023), who reported moisture, protein, fat, fiber, and ash contents in house crickets of 12.64%, 60.40%, 16.92%, 12.93%, and 3.81%, respectively. Consequently, crickets are regarded as extremely nutrient-dense insects, particularly notable for their protein, fat, vitamins, and minerals (Ayieko et al., 2016; Stull et al., 2018; Kiiru et al., 2025). However, the protein content in crickets can vary widely, from 13% to 77% of dry matter, depending on factors such as species, developmental stage, and cultivation methods (wild-caught versus farm-raised) (Kouřimská and Adámková, 2016; Zhou et al., 2022). Currently, insect consumption remains a niche market worldwide, although it is expected to increase steadily. Emphasizing the nutritional and environmental benefits of insects rather than their often unappetizing image may increase consumer acceptance (Hartmann and Siegrist, 2017; Sogari et al., 2019). This is vital, as some consumers initially find insect-based foods repulsive. Most insect-based foods available on the market today such as snacks, cookies, cakes, milk, burgers and granola are processed to conceal any visible traces of insects (Kauppi et al., 2019). Additionally, attractive packaging designs aim to entice consumers. One effective approach to improve the perception of crickets and enhance their palatability is to dry and grind them into flour. Incorporating cricket flour as an ingredient allows food products to increase protein content without the visual presence of whole insects. Examples of such products include cricket rice powder, cricket crackers, cricket cookies, and cricket-based bakery items.

Production of cricket protein hydrolysate is one effective method to utilize the protein content in crickets. Protein hydrolysate is a product derived from edible proteins through protein hydrolysis, which involves enzymatic or microbiological fermentation, followed by separation and purification. Enzymatic hydrolysis is widely used in the food industry because it employs natural enzymes primarily proteases such as papain from papaya and bromelain from pineapple to break down proteins efficiently. These enzymes are preferred due to their effectiveness and safety (Zhang et al., 2019; Siddik et al., 2021).

During hydrolysis, proteins undergo structural changes, resulting in free amino groups, carbonyl groups, and sulfur-containing functional groups. These modifications confer specific functional properties valuable in food processing, including emulsification, foaming, gelation, antioxidation, antibacterial activity, flavor enhancement, and anti-freezing effects (Tang et al., 2023). Compared to hydrolysis using acid or alkali treatments, enzymatic hydrolysis is considered the most effective technique. This is because the protease enzymes act in a selective manner depending on the type of protease employed, producing peptides with specific amino acid compositions and sequences (Bahri et al., 2021). Bromelain, for example, is a complex mixture of proteolytic enzymes extracted from the pineapple plant. The stem, fruit, and crown of the pineapple plant are rich sources of bromelain (Liu et al., 2017; Johny et al., 2022). However, the pineapple storage process can affect the characteristics of the crude bromelain produced, both in terms of yield, protein content, enzyme activity, and bromelain content. The longer the pineapple was stored, the greater the crude bromelain yield obtained. While the yield increases, the levels of protein content, bromelain content, and crude bromelain activity decrease. From day 0 to day 9, crude bromelain production in pineapple storage increased from 1.80% to 2.30%. Protein content decreased from 18.76 to 6.02g/mL, crude bromelain activity level decreased from 73.73U/mg to 27.60U/mg, and bromelain content decreased from 2.2435mg/mL to 0.5175mg/mL. Therefore, the pineapples that have not been sold for 9 days can still be utilized. Pineapples that are nearing spoilage can still be processed into bromelain (Nanda et al., 2025).

Protein hydrolysates using bromelain enzymes have been extensively studied between 2020 and 2025. For instance, Nurdiani et al. (2024) investigated protein hydrolysate from *Pangasius* and found that using 0.04% bromelain enzyme for 2.8 hours resulted in a degree of hydrolysis (DH) of 35.88%, a pH of 7.07, and antioxidant activity of 29.86%, along with elevated levels of glycine, L-glutamic acid, and L-aspartic acid. Similarly, Bahri et al. (2021) studied protein hydrolysate derived from tofu dregs and reported optimal conditions of 11% crude bromelain extract for 120min, achieving a DH of 22.82%. In the study by Parhusip et al. (2024), protein hydrolysate production from overripe tempeh using 25% crude bromelain yielded the highest total amino acid content (36,682.002mg/100g), with prolonged fermentation time and higher enzyme concentrations significantly increasing protein content (45.96 to 50.87%) and water-soluble amino acid content (14.55 to 39.86%). Priyanto et al. (2022) examined protein hydrolysate from apple snails (*Pila ampullacea*) and found the highest protein yield using bromelain at concentrations of 1%–10% for durations of 12 to 15 hours. Furthermore, Puspitasari et al. (2022) optimized hydrolysis of apple snail protein with 15% bromelain for 18 hours, resulting in a DH of 72.09%, soluble protein content of 9.03%, total peptides of 10.84mg/mL, yield of 68.16%, and glutamic acid concentration of 107.47ppm.

This study aims to determine the physicochemical properties of cricket protein hydrolysate by evaluating the effects of digestion time and bromelain concentration. The optimal conditions identified will be applied to produce a seasoning sauce from cricket protein hydrolysate that mimics commercial oyster sauce. This cricket-based sauce product is expected to enhance the economic value of crickets by offering a seasoning alternative with higher protein content and reduced salt levels, thus catering to consumers seeking healthier, low-sodium food options.

MATERIALS & METHODS

Cricket Preparation

Crickets that did not meet the desired size for sale were obtained from Bang Rabang Cricket Farm, located in Phra Nakhon Si Ayutthaya Province, Thailand. The crickets were washed three times with clean water, then blanched in boiling water at 100°C for 1 minute before being drained on a wire rack. Subsequently, the crickets were stored in polypropylene bags, each weighing 0.5 kg and kept at -14°C until analysis. An image of the crickets is presented in Fig. 1.

Fig. 1: Crickets sourced from a farm in Thailand.

Proximate Composition Analysis of Crickets

The crickets were finely ground using a food processor (SEVERIN model SEV-3865) and analyzed for proximate composition, including moisture, ash, crude protein, crude fat, and crude fiber, following AOAC methods (AOAC, 2019). Total carbohydrate content was calculated by subtracting the sum of moisture, fat, protein, and ash contents from 100.

Selection of Reference Oyster Sauce

Commercial oyster sauces from five different brands, available in Thai department stores, were randomly selected for sensory evaluation. Attributes assessed included color, odor, taste, texture, and overall acceptability. Fifty untrained panelists (aged 18-60 years, with no history of allergies to the ingredients) participated using a 9-point hedonic scale. For sample preparation, 10 g of water spinach was stir-fried with 2 g of oyster sauce from each brand. A pan was heated to medium heat, and 10 g of vegetable oil was added. Once hot, the water spinach was introduced, followed by approximately 20 g of water and seasoning with oyster sauce. The mixture was stir-fried for 15-20 seconds at medium heat. Panelists received 50 g samples served on plates coded with randomized 3-digit numbers to prevent bias and were provided with drinking water to cleanse their palate between tastings.

Determination of Optimum Bromelain Concentration and Digestion Time for Extraction of Cricket Protein Hydrolysate

100 g of finely ground crickets were mixed with water at a ratio of 1:2 (w/v). Bromelain enzyme was then added at four different concentrations: 0, 0.25, 0.5, and 0.75% (w/w). The mixtures were thoroughly blended and incubated at 55±1°C for varying durations of 0, 2, 4, 6, and 8 hours. Throughout the incubation, the container lids were kept closed to prevent contamination and evaporation. After incubation, enzymatic activity was halted by heating the mixtures at 100°C for 3 minutes. Subsequently, the hydrolysates were filtered using a 400-mesh nylon filter. The filtered cricket protein hydrolysates were transferred into sterilized glass bottles, tightly sealed, and stored in a refrigerator at temperatures between 4°C and 7°C. The protein content of each hydrolysate sample was analyzed following the AOAC method (AOAC, 2019). The optimum bromelain concentration and digestion time were selected based on the treatment that yielded the highest protein content. These optimum extraction conditions were then applied to produce cricket protein hydrolysate for use as the main ingredient in the development of cricket sauce products designed to mimic commercial oyster sauce in subsequent experiments.

Development of Cricket Sauce

A mixture design approach was employed to determine the ideal formulation for cricket sauce production. Specifically, a constrained simplex lattice mixture design was used to optimize the quantities of three key components: cricket protein hydrolysate (X1: 55-60%), salt (X2: 0-5%), and low-sodium soy sauce (X3: 0-10%). Seven different recipes were formulated and tested, while the quantities of all other ingredients in the recipe were kept constant (Table 1). In subsequent sections, the seven recipes of cricket sauce production are denoted as CSR1, CSR2, CSR3, CSR4, CSR5, and CSR6.

Table 1: Ingredient optimization for cricket sauce

Ingredient (%)	Recipes						
	CSR1	CSR2	CSR3	CSR4	CSR5	CSR6	CSR7
Cricket protein hydrolysate	59.2	55	56.6	60	58.3	59.2	60
Salt	4.2	5	4.2	5	3.3	1.6	0
Low sodium soy sauce	1.6	5	4.2	0	3.3	4.2	5
Water (for dissolve starch)	15	15	15	15	15	15	15
Sugar	14	14	14	14	14	14	14
Modified starch	2.85	2.85	2.85	2.85	2.85	2.85	2.85
Monosodium glutamates	2.43	2.43	2.43	2.43	2.43	2.43	2.43
Black sweet soy sauce	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Caramel	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Citric acid	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Total	100	100	100	100	100	100	100

Production of Cricket Sauces

All cricket sauces in this study were prepared using the formulations listed in Table 1. Initially, all dry ingredients, including sugar, salt, MSG, and citric acid were thoroughly mixed together. Separately, all liquid ingredients including low-sodium soy sauce, sweet soy sauce, and caramel were combined, and modified tapioca starch was dissolved in water to form a slurry. The cricket protein hydrolysate was subjected to heat treatment until the temperature of the mixture reached 75-80°C. The prepared dry ingredients were then added to the heated protein hydrolysate and stirred continuously until fully dissolved, resulting in a smooth texture. Subsequently, the liquid ingredients were incorporated into the mixture and stirred again until smooth. While stirring continuously, the tapioca starch slurry was gradually added to the mixture. After completing the addition, the temperature was raised to 95-100°C and the mixture was stirred continuously for 5 minutes. The hot cricket sauce mixture was then transferred into sterilized containers and sealed tightly. The bottled sauce underwent a heat treatment at 95-100°C for 10 minutes. Following heat treatment, cold air was applied to rapidly cool the bottles, which were then stored at room temperature (30-35°C). The physicochemical properties of the cricket sauces were subsequently analyzed.

Analysis of Physicochemical Properties of Cricket Sauce

The physicochemical properties of the cricket sauce samples were measured using several analytical techniques. Color values were determined with a colorimeter (Hunter Lab model AMT 501, USA) and expressed according to the Commission Internationale de l'Éclairage (CIE) chromaticity coordinates: lightness (L*), redness (a*), and yellowness (b*). Viscosity was analyzed using a viscometer (Brookfield model DV2T), while total soluble solids (°Brix) were measured with a hand refractometer (Atago model MASTER-53M). pH values were recorded using a pH meter (Hanna model HI2202), and sodium content was determined by a salt content (NaCl) refractometer (Hanna model HI96821). Additionally, proximate composition including moisture, ash, crude protein, crude fat, crude fiber, and carbohydrate contents was analyzed following AOAC standard methods (AOAC, 2019).

Sensory Evaluation of Cricket Sauce

Sensory evaluation of the cricket sauce was conducted to assess color, odor, taste, texture, and overall liking using a 9-point hedonic scale, where 1 represents "dislike extremely" and 9 represents "like extremely." A total of 50 untrained panelists, aged 18 to 60 years and with no history of allergies to the ingredients, participated in the evaluation. The average rating for each sensory attribute was calculated, and the most acceptable cricket sauce formulation was selected for subsequent shelf-life studies.

Study of Shelf Life of Cricket Sauce

The cricket sauce was filled into sterilized glass bottles and stored at ambient temperatures of 30-35°C. Physicochemical properties including color, viscosity, total soluble solids, pH, and sodium content were analyzed at two-week intervals over a three-month period. Microbiological analyses were conducted according to the Food and Drug Administration's Bacteriological Analytical Manual (BAM). These analyses included total plate count, yeast and mold counts (YMCs), *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp. Colony-forming units (CFUs) were counted on dilution plates, averaged, and reported as CFU/g for total plate counts and YMCs, and as CFU/25 g for *E. coli*, *S. aureus*, and *Salmonella* spp. Microbiological results were compared against the criteria established by the Thai Industrial Standard (TIS 1317-2538).

Statistical Analysis

All results are expressed as mean±SD, obtained from at least three independent trials. Data were statistically analyzed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test to determine significant differences. Differences were considered statistically significant at P≤0.05. Statistical analyses were performed using SPSS Statistics software, version 18.0.

RESULTS & DISCUSSION

Proximate Composition of Fresh Crickets

The proximate composition of fresh crickets was analyzed and found to contain 65.58% moisture, 1.17% ash, 19.11% crude protein, 3.57% crude fat, 2.44% crude fiber, and 8.13% total carbohydrates (Table 2). These results indicated that protein was the major nutrient component of the crickets. Several studies have reported comparable protein and fat contents in different cricket species. For instance, Chooklin et al. (2025) found that Sading powder (*Acheta domestica*) contained 59.37% protein and 16.78% fat, while Thongdam powder (*Gryllus bimaculatus*) contained 55.47% protein and 17.01% fat. Udomsil et al. (2019) similarly reported protein contents of 60–70% dry weight and fat between 10.4% and 23.4% dry weight for both *A. domesticus* and *G. bimaculatus*. Laroche et al. (2019) found 53.5% protein in Sading powder (*A. domestica*), and Rumpold and Schlüter (2013) reported protein contents ranging from 55% to 70% in *A. domesticus* meal. Montowska et al. (2019) observed 42.0–45.8% protein and 23.6–29.1% fat in cricket powder (*A. domesticus*). Kouřimská and Adámková (2016) reported a wide range of protein contents in crickets, from 13% to 77% of dry matter. Overall, the proximate composition analysis confirmed that protein constitutes the primary nutritional component of crickets, emphasizing their potential as an alternative protein source. However, the exact composition depends on species, developmental stage, and whether the crickets were wild-caught or farm-raised.

Table 2: Proximate composition of fresh crickets

Proximate composition	Value (wet basis)
Moisture (%)	65.58±0.09
Ash (%)	1.17±0.18
Crude protein (%)	19.11±0.13
Crude fat (%)	3.57±0.14
Total carbohydrate (%)	2.44±0.22

Selection of Prototype Oyster Sauce Product

Commercial oyster sauces from five different brands were evaluated for sensory acceptance. Brand 5 oyster sauce received the highest ratings in terms of taste, odor, and overall acceptance, with mean scores of 6.88, 6.97, and 6.92, respectively (P≤0.05). However, the ratings for color did not differ significantly among the brands (Table 3). The oyster sauces exhibited a total protein content of 1.10%, viscosity of 22,840 cPs, pH of 4.27, total acidity of 0.167%, total soluble solids of 30 °Brix, and salt content of 1,694mg (4.3%). Its color values were lightness (L*) of 13.26, redness (a*) of 9.56, and yellowness (b*) of -4.39 (Table 4). Brand 5 oyster sauce was selected as the prototype for comparison with the cricket sauce developed in this study. This selection considered the panelists' preferences.

Table 3: Means hedonic score of sensory evaluation of commercial oyster sauces

Commercial	Hedonic score			
oyster sauces	Color^{ns}	Odor	Taste	Overall acceptability
Brand 1	6.39±1.52	6.23±1.63^{b}	6.21±1.81^{b}	6.35±0.93^{c}
Brand 2	6.27±1.15	6.45±1.42^{b}	6.25±1.72^{b}	6.23±1.12^{c}
Brand 3	6.21±1.49	6.18±1.38^{c}	6.11±1.79^{c}	6.63±1.35^{b}
Brand 4	6.31±1.65	6.32±1.48^{b}	6.33±1.64^{b}	6.57±0.95^{ab}
Brand 5	6.39±1.65	6.88±1.48^{a}	6.97±1.64^{a}	6.92±0.95^{a}

Values (Mean±SD) bearing different letters in the same column are significantly different (P≤0.05).

Table 4: Physicochemical properties of a selected prototype oyster sauce product

Physicochemical properties		Value
Protein content (%)		1.10±0.13
Color value	L*; lightness	13.26±0.27
	a*; redness	9.56±0.56
	b*; yellowness	-4.39±0.20
Viscosity; centipoise; cPs		9,710±2.03
pH		4.27±0.07
Total acidity; %		0.167±0.12
Total soluble solid; °Brix		30.0±0.00
Sodium content; mg		1,694±0.38
Sodium content; %		4.30±0.06

The Optimum Bromelain Concentration and Digestion Time for Extraction of Cricket Protein

The protein content of cricket protein hydrolysate produced using varying bromelain concentrations and digestion times proved that both enzyme concentration and digestion time significantly affected the amount of protein extracted (P≤0.05) (Table 5). Protein content ranged from 5.92% to 9.36% at 0% bromelain concentration, 11.58 to 20.71% at 0.25%, 13.36 to 21.87% at 0.5%, and 14.98 to 22.96% at 0.75%, measured over digestion times from 0 to 8 hours. The amount of protein successfully broken down from the total protein was indicated by the soluble protein content, which was measured by analyzing the hydrolyzed sample. A higher soluble protein content value reflects a more efficient hydrolysis process. The treatment using 0.75% bromelain enzyme with a digestion time of 6 hours resulted in the highest total protein content of 22.96%. This was closely followed by the treatment with 0.5% bromelain for 6 hours, which produced 21.87% protein. Conversely, the lowest protein content values of 5.92 and 5.96% were observed in treatments without enzyme (0% bromelain) at digestion times of 2 and 8 hours, respectively. Based on these findings, the bromelain concentration of 0.75% combined with a digestion time of 6 hours was selected as the optimum condition for extracting cricket protein hydrolysate. This optimized hydrolysate was subsequently used as the primary ingredient in the production of cricket sauce, following these steps.

The optimum condition for cricket protein extraction was found to be 0.75% bromelain concentration with 6 hours of digestion time, resulting in a protein hydrolysate containing 22.96% protein. The protein content of the hydrolysate increased as both enzyme concentration and digestion time increased. Bromelain is a protease enzyme belonging to the hydrolase family that catalyzes the cleavage of peptide bonds in proteins, thereby modifying the amino acid structure and producing peptides with variable properties and molecular weights (Varilla et al., 2021; Chakraborty et al., 2021). Under constant substrate concentration and digestion conditions, enzyme concentration and digestion time are two critical factors influencing protein hydrolysis. The reaction rate initially rises with increasing enzyme concentration but tends to stabilize or even gradually decrease at high enzyme levels, as the amount of substrate protein diminishes and is converted into free amino acids or peptides (Switzar et al., 2013; Deng et al., 2018; Arteaga et al., 2020). Protein digestion produces amino acids and dipeptides; some of these digestion products are stable and do not require further hydrolysis. These dipeptides and amino acids are important contributors to the flavor and overall pleasant taste of protein hydrolysates. Moreover, the protein content of the raw material influences the required digestion time. A higher protein content generally necessitates longer digestion. As the substrate protein is depleted and enzyme concentration exceeds substrate availability, the hydrolysis process stabilizes and slows down, producing mainly free amino acids and peptides (Sousa et al., 2020; Qing et al., 2022). The high protein content observed in this study suggests that bromelain effectively catalyzed the hydrolysis process. Increasing the enzyme concentration accelerated the reaction, thereby enhancing the protein hydrolysate yield. Bromelain enzymes specifically break down peptide bonds in proteins into simpler amino acids, which improves digestibility and potentially the bioavailability of the protein hydrolysate (Zarei et al., 2012).

Table 5: Protein content of cricket protein extracted using enzyme extraction

Treatment	Bromelain concentration (%)	Digestion time (h)	Protein content (%)
1	0	0	7.30±0.10^{q}
2	0	2	5.92±0.12^{r}
3	0	4	7.58±0.10^{p}
4	0	6	9.36±0.09^{o}
5	0	8	5.96±0.06^{r}
6	0.25	0	11.58±0.09^{n}
7	0.25	2	14.77±0.08^{l}
8	0.25	4	20.71±0.12^{e}
9	0.25	6	19.45±0.11^{g}
10	0.25	8	18.18±0.04^{i}
11	0.5	0	13.61±0.11^{m}
12	0.5	2	16.15±0.15^{j}
13	0.5	4	19.33±0.17^{g}
14	0.5	6	21.87±0.10^{b}
15	0.5	8	21.58±0.06^{c}
16	0.75	0	14.98±0.14^{k}
17	0.75	2	18.80±0.90^{h}
18	0.75	4	19.94±0.15^{f}
19	0.75	6	22.96±0.16^{a}
20	0.75	8	21.22±0.10^{d}

$^{a-k}$ Values with different letters in the same column are significantly different (P≤0.05).

The protein content obtained in this study was higher than that reported by Priyanto and Trisna (2022), who found that apple snail (*P.ampullaceal*) protein hydrolysates produced using 1–10% bromelain enzyme over 12 to 15 hours yielded the highest soluble protein content of 3.35 to 4.18%. However, the results were lower than those of Puspitasari et al. (2022), who applied 15% bromelain enzyme for 18 hours of digestion and achieved a degree of hydrolysis (DH) of 72.09% and soluble protein content of 9.03% from apple snails (*P. ampullaceal*). Parhusip et al. (2024) reported that fermenting overripe tempeh for 5 days with 0.25% bromelain enzyme resulted in the highest crude protein content of 49.10%. Furthermore, Bahri et al. (2021) studied protein hydrolysate production from tofu dregs and found the optimum condition using 11% crude bromelain extract for 120min, achieving a degree of hydrolysis of 22.82%.

Various studies indicate that differences in initial protein content of raw materials, the types and sources of enzymes used, as well as their efficacy, can all contribute to discrepancies observed in these results. Both enzyme type and efficacy influence the extent to which protein is solubilized and broken down, affecting the degree of hydrolysis (DH), while peptide solubility also influences the measured soluble protein content in hydrolysates. Generally, the concentration of soluble protein increases proportionally with the degree of hydrolysis and enhanced peptide solubility (Ovissipour et al., 2012).

Physicochemical Properties of Cricket Sauce

After being stored for one day, all seven cricket sauce formulations each varying in the proportions of cricket protein hydrolysate, salt, and low-sodium soy sauce exhibited a dark brown color and a salty taste with a characteristic soy sauce aroma. The texture of the sauces was thick but not sticky. When scooped and poured with a spoon, the sauce flowed continuously and smoothly. The appearance of the cricket sauces corresponding to all seven recipes is shown in Fig. 2.

Fig. 2: The cricket sauce of all seven recipes.

The color analysis revealed that the amounts of protein, salt, and low sodium soy sauce significantly affected the lightness (L*) and redness (a*) of the cricket sauce ($P \leq 0.05$) but did not have a statistically significant effect on yellowness (b*) ($P > 0.05$). Recipes CSR3, CSR5, and CSR7 exhibited the highest lightness values of 4.65, 4.57, and 4.87, respectively, while CSR1 showed the lowest lightness value of 2.53. CSR7 had the highest redness value of 13.85; however, its redness was not significantly different from those of CSR1, CSR3, and CSR5 (9.21, 9.92, and 12.82, respectively). CSR2 showed the lowest redness value of 6.52. The color values of all seven cricket sauce recipes are illustrated in Fig. 3.

The physicochemical properties of the seven cricket sauce recipes (Table 6) indicate that the amounts of cricket protein hydrolysate, salt, and low-sodium soy sauce significantly affected the salt content, viscosity, pH, and total soluble solids of the cricket sauces ($P \leq 0.05$). CSR2 exhibited the highest salt content at 1,412mg (3.56%), whereas CSR5 showed the lowest salt content at 442mg (1.12%). Regarding viscosity, CSR1 had the highest value of 2,412.3 cPs, with viscosity across all seven recipes ranging from 1,166.7 to 2,412.3 cPs. The highest pH value of 5.62 was observed in CSR5, while the pH levels of all recipes ranged between 5.29 and 5.38. Additionally, CSR2 had the highest total soluble solids content at 33.60 °Brix, and CSR5 recorded the lowest at 27.70 °Brix.

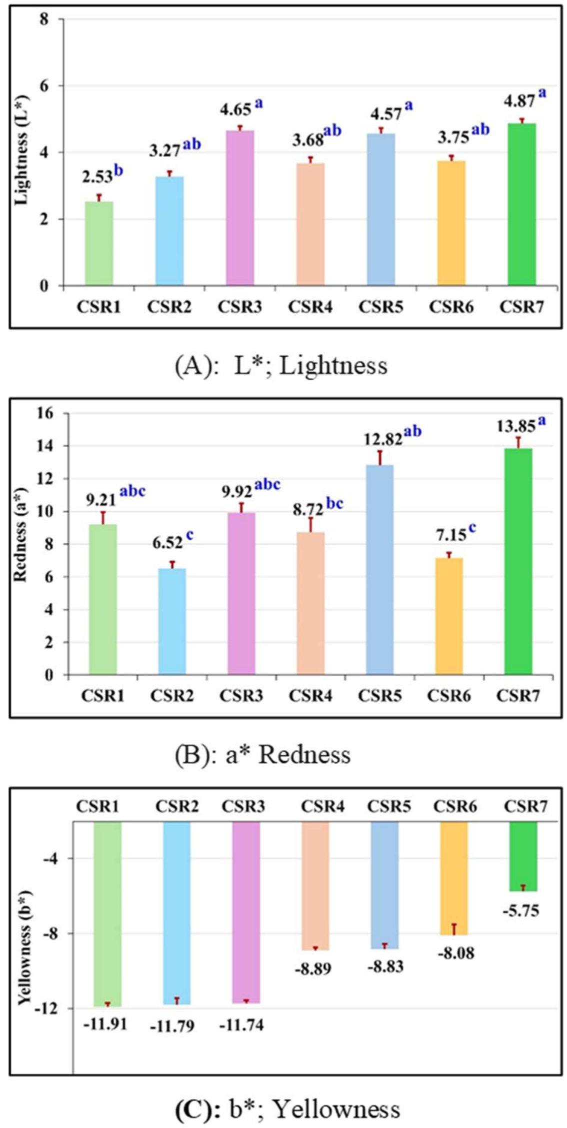

Fig. 3: The color value of seven cricket recipes (formulations shown in Table 1); a-c: bar sharing different letters are significantly different ($P \leq 0.05$).

Table 6: Physicochemical properties of cricket sauce

Recipes	Physicochemical properties				
	Viscosity (cPs)	Total soluble solid (°Brix)	pH	Sodium content (mg)	Sodium content (%)
1 (CSR1)	2,412.3±2.30^{a}	30.43±0.15^{e}	5.39±0.02^{c}	780±0.15^{e}	1.99±0.19^{e}
2 (CSR2)	2,214.0±1.40^{b}	33.60±0.10^{a}	5.23±0.02^{c}	1,412±0.17^{a}	3.56±0.16^{a}
3 (CSR3)	1,839.6±7.79^{c}	31.10±0.15^{cd}	5.29±0.01^{d}	1,322±0.14^{b}	3.40±0.14^{b}
4 (CSR4)	2,169.3±6.44^{b}	32.96±0.10^{b}	5.32±0.01^{c}	1,335±0.16^{b}	3.40±0.12^{b}
5 (CSR5)	1,392.0±4.60^{d}	27.70±0.13^{f}	5.62±0.02^{a}	442±0.15^{f}	1.12±0.11^{f}
6 (CSR6)	1,414.6±2.01^{d}	31.43±0.16^{c}	5.38±0.01^{b}	1,106±0.16^{d}	2.81±0.18^{d}
7 (CSR7)	1,166.7±1.53^{e}	30.53±0.12^{de}	5.38±0.01^{b}	1,219±0.13^{c}	3.10±0.13^{c}

a-g Values with different letters in the same column are significantly different ($P \leq 0.05$).

Table 7: Proximate composition of cricket sauce

Recipes	Proximate composition; %					
	Moisture	Ash	Protein	Fat	Fiber	Carbohydrate
1 (CSR1)	69.72±0.27[b]	3.41±0.16[d]	5.15±0.02[a]	0.51±0.01[cd]	0.69±0.51[a]	20.49±0.18[b]
2 (CSR2)	64.94±0.44[d]	7.44±0.14[a]	4.36±0.03[c]	0.43±0.01[f]	0.36±0.90[b]	22.45±0.33[a]
3 (CSR3)	67.21±0.29[c]	7.32±0.25[a]	4.51±0.13[b]	0.46±0.02[ef]	0.47±0.39[b]	20.00±0.12[b]
4 (CSR4)	67.56±0.28[c]	7.24±0.12[a]	4.35±0.03[c]	0.61±0.01[a]	0.75±0.51[a]	19.47±0.33[b]
5 (CSR5)	71.82±0.51[a]	2.05±0.44[e]	4.36±0.01[c]	0.53±0.02[c]	0.66±0.50[a]	20.55±0.46[b]
6 (CSR6)	68.64±0.97[bc]	5.66±0.14[c]	4.55±0.02[b]	0.49±0.02[de]	0.69±0.10[a]	19.94±0.80[b]
7 (CSR7)	68.24±0.69[c]	6.35±0.18[b]	4.31±0.01[c]	0.56±0.02[b]	0.78±0.51[a]	19.73±0.61[b]

Values (mean±SD) bearing different letters in the same column are significantly different (P≤0.05).

Proximate Composition of Cricket Sauce

The proximate composition of the seven cricket sauce recipes (Table 7) showed that the levels of cricket protein hydrolysate, salt, and low sodium soy sauce significantly influenced moisture, ash, crude protein, crude fat, crude fiber, and carbohydrate contents (P≤0.05). CSR5 exhibited the highest moisture content at 71.82%, with moisture across all recipes ranging from 64.94% to 71.82%. CSR4 showed the highest crude fat content of 0.61%, whereas CSR2 had the lowest at 0.43%. Regarding crude protein, CSR1 presented the highest content of 5.15%, with protein levels across recipes ranging between 4.31% and 5.15%. For crude fiber and carbohydrates, CSR1 contained the highest fiber content of 0.69%, and CSR2 had the highest carbohydrate content at 22.45%. The fiber contents ranged from 0.36% to 0.69%, while carbohydrate contents ranged from 19.47% to 22.45% across all seven recipes.

The results indicated that CSR6, made with 59.2% cricket protein hydrolysate, 1.7% salt, and 4.2% low-sodium soy sauce, all seven recipes using varying concentrations of protein hydrolysate, salt, and low-sodium soy sauce was thick, tasted sweeter than regular oyster sauce, and looked dark brown because it included caramel. It also showed a smell and taste similar to cricket protein hydrolysate. It also showed a smell and taste similar to cricket protein hydrolysate. The ingredients used made the cricket sauce taste sweeter by lowering the salt content, which highlighted other flavors, particularly sweetness. Key features of cricket sauce were its high protein level (up to 5.15% from cricket protein hydrolysate, low salt level (1,106mg, 2.81%), pH (5.38), and viscosity (1,414.6 cPs). In contrast, the reference oyster sauce had a pH of 4.27, a viscosity of 9,823 cPs, a protein content of 1.10%, and a salt content of 1,694mg (4.30%). This study created the cricket sauce to replicate the characteristics of oyster sauce by using comparable enzymatic digestion and production ingredients. Therefore the oyster sauce physicochemical standard (TIS 1317-2538) was applied for comparison. The physicochemical characteristics of all seven cricket sauce recipes were found to meet the required standards, particularly the sodium content (sodium chloride concentration), which ranged from 1.12 to 3.40%. Its sodium content was lower than commercial oyster sauce, which has a sodium content of up to 4.30% and 1,694mg and the standard for oyster sauce, which stipulates that the salt content of oyster sauce must not be less than 13%. As cricket protein isolate was used as the main ingredient in the production of cricket sauce, it was found that the protein content of all seven cricket sauce recipes ranged between 2.29% and 2.36%, while the reference oyster sauce had a protein content of only 0.52%. Therefore, the cricket sauce developed in this study had a higher protein content and lower sodium content than commercial oyster sauce. Due to cricket protein isolate was the primary ingredient used to make cricket sauce, it was discovered that all seven cricket sauce recipes had protein contents ranging from 4.36% to 5.15%, whereas the reference oyster sauce only had 1.10% protein. As a result, compared to commercial oyster sauce, the cricket sauce created in this study had a higher protein content and a lower sodium content.

Sensory Evaluation of Cricket Sauce

Sensory evaluation conducted with 50 panelists on seven cricket sauce recipes revealed that the varying amounts of cricket protein hydrolysate, salt, and low sodium soy sauce did not significantly affect the ratings for color and odor (P>0.05). However, these factors significantly influenced taste, texture, and overall acceptability (P≤0.05) (Table 8). Among the recipes, CSR6 received the highest ratings across all attributes, with scores of 7.38 for color, 6.90 for odor, 6.92 for taste, 7.22 for texture, and 7.00 for overall acceptability. These ratings correspond to a liking level between "like" and "moderately like". Therefore, CSR6, formulated with cricket protein extract, salt, and low sodium soy sauce at proportions of 59.2%, 1.7%, and 4.2%, respectively, was selected as the optimal recipe for subsequent shelf life studies.

Table 8: Means hedonic score of sensory evaluation of cricket sauce

Recipes	Hedonic score				
	Color	Odor	Taste	Texture	Overall acceptability
1 (CSR1)	6.88±1.74	6.62±1.86	6.34±1.95[b]	7.12±1.75[a]	6.78±1.69[ab]
2 (CSR2)	6.82±1.57	6.66±1.88	6.24±1.88[b]	6.60±1.90[ab]	6.52±1.71[ab]
3 (CSR3)	6.96±1.58	6.11±1.81	6.20±1.87[b]	6.70±1.64[ab]	6.82±1.66[ab]
4 (CSR4)	6.66±1.89	6.62±2.06	6.28±1.96[b]	6.54±1.79[ab]	6.33±1.75[ab]
5 (CSR5)	6.72±1.66	6.48±2.02	6.18±1.79[b]	6.96±1.76[ab]	6.58±1.59[ab]
6 (CSR6)	7.38±2.21	6.90±2.02	6.92±1.89[a]	7.22±1.87[a]	7.00±1.91[a]
7 (CSR7)	6.78±1.74	6.69±1.74	6.58±1.77[b]	6.30±1.64[b]	6.18±1.75[b]

Values (mean±SD) bearing different letters in the same column are significantly different (P≤0.05).

Determination of Shelf Life of Cricket Sauce

Cricket sauce prepared using CSR6 (consisting of 59.2% cricket protein hydrolysate, 1.7% salt, and 4.2% low sodium soy sauce) was stored at 35°C for three months. During this period, pH, total soluble solids, and salt content were all significantly affected (P≤0.05) (Fig. 4). Throughout the storage duration, the pH ranged narrowly between 5.42 and 5.44. Salt content remained stable, fluctuating between 1,013mg and 1,089mg (2.56% to 2.77%), while total soluble solids varied slightly from 30 °Brix to 31 °Brix. Viscosity showed an increasing trend over the storage period, ranging from 1,230 cPs to 1,894 cPs. This variation may be attributed to complex formation between protein molecules

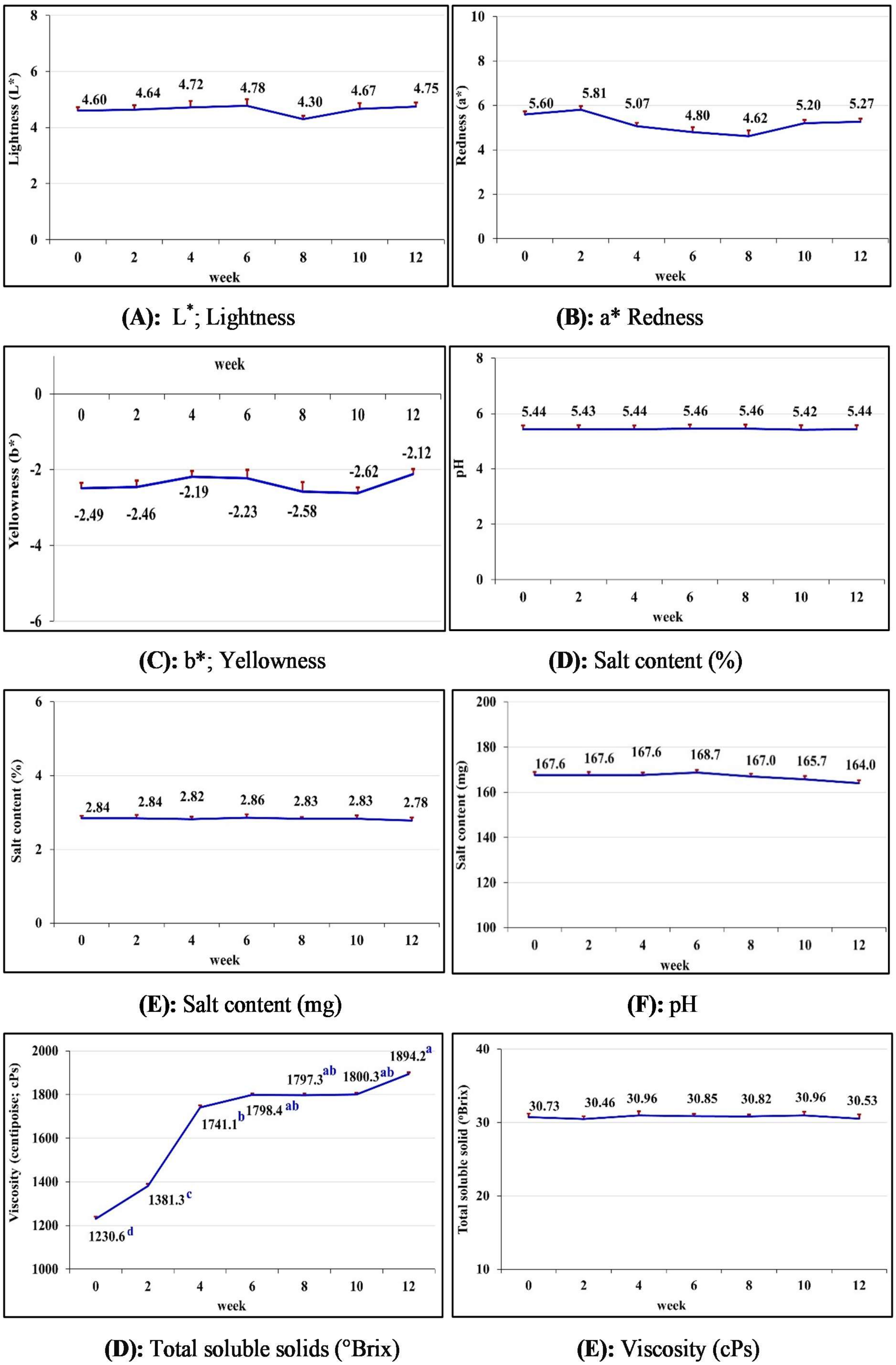

Fig. 4: Physicochemical properties of cricket sauce during storage three months; a-c: line sharing different letters are significantly different (P≤0.05).

and polysaccharide starch molecules. Polysaccharides potentially influence viscosity by adsorbing protein molecules onto their surfaces, thereby altering the rheological properties of the sauce (De Kruif and Tuinier, 2001; Ahouagi et al., 2021; Fernández et al., 2022). Additionally, heat-induced structural changes to constituents such as pectin gelatin may result in a stiffer matrix that increases flow resistance within the sample. Therefore, to improve the overall texture of sauces with high solids content, the incorporation of additional thickeners is often necessary (Diantom et al., 2017). However, the pH, salt content, and viscosity values were found to be within the standard criteria, which stipulates that oyster sauce must have a pH of at least 4.4, a salt content of at least 13%, and a viscosity of at least 18,000 cPs when compared to the Thai Industrial Standard for Oyster Sauce (TIS 1317-2538).

The microbiological analysis of cricket sauce stored at 35°C revealed a total microbial count not exceeding 1×10^4 CFU/g. The counts of coliform bacteria were less than 3 CFU/g, and yeast and mold counts were under 10 CFU/g. No growth of *Escherichia coli*, *Staphylococcus aureus*, or *Salmonella* spp. was detected throughout the storage period. Comparison of the cricket sauce's physicochemical properties with the Thai Industrial Standard for Oyster Sauce (TIS 1317-2538) revealed that its pH value complied with the minimum required level of 4.4. However, both the salt content and viscosity were lower than the standard thresholds of 13% salt content and 18,000 cPs viscosity.

The microbiological properties of the cricket sauce complied with the requirements of the Thai Industrial Standard for Oyster Sauce (TIS 1317-2538), which specifies that the total microbial count must be less than 1,000 CFU/g, total yeast and mold must be less than 10 CFU/g, the most probable number (MPN) of coliform organisms must be less than 3 CFU/g and no presence of *Salmonella*, *Staphylococcus aureus*, or *Escherichia coli* should be detected. The cricket sauce developed in this study exhibited higher protein content and lower salt content compared to commercial oyster sauces, owing to the inclusion of cricket protein hydrolysate and low-sodium soy sauce. Therefore, this product is suitable for consumers who prefer reduced-sodium seasoning or are health-conscious.

Conclusion

Edible crickets are highly regarded as a novel food source with great potential to contribute to global food security. They are rich in protein and contain appreciable amounts of essential mineral elements such as calcium, potassium, magnesium, phosphorus, sodium, iron, zinc, manganese, and copper. The proximate composition of cricket flesh analyzed in this study comprised 19.11% crude protein, 3.57% crude fat, 2.44% crude fiber, 1.17% total ash, 65.58% moisture, and 8.13% total carbohydrates. The protein content of cricket protein hydrolysate was significantly influenced by the concentration of the bromelain enzyme and the digestion time. Increasing both the bromelain concentration and digestion duration resulted in higher protein content in the hydrolysate. Specifically, digestion with 0.75% bromelain for 6 hours yielded the highest protein content of 22.96%. Over the past 10 to 20 years, the production of protein hydrolysates using enzymatic hydrolysis has expanded substantially due to their capacity to modulate texture and functionality of food products including emulsifying, foaming, and gelation properties. Protein hydrolysates can engage in various chemical reactions with other ingredients, thereby enhancing the nutritional, physicochemical, and sensory attributes of food formulation. In this research, cricket protein hydrolysate was successfully used as the primary ingredient in the formulation of cricket sauce, which proved physicochemical properties and a production process closely resembling commercial oyster sauce. The optimal formulation comprised 59.2% cricket protein hydrolysate, 1.7% salt, and 4.2% low-sodium soy sauce. Remarkably, the resulting cricket sauce product exhibited higher protein content compared to commercial oyster sauce.

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Data Availability: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Statement: This study did not require ethical approval, as it did not involve sensitive human data or animal subjects. Untrained panelists participated in the sensory evaluation. All participants were informed about the test's purpose and voluntarily agreed to participate, and no personal identifying information was collected.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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