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RESEARCH ARTICLE

Nutrient profiles and browning control of wasp larvae

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Abstract

Entomophagy, the practice of consuming insects, has long been recognized as a sustainable and renewable source of food. This study aimed at assessing the nutritional value of three species of wasp larvae (*Provespa barthelemyi*, *Vespa mandarinia*, and *V. velutina*) and explore effective strategies to address enzymatic browning during processing. The study reveals that wasp larvae exhibit considerable potential as a dietary resource, primarily due to their high protein content, more than 50% of the total dry matter. Remarkably, the presence of vitamin B_2 in wasp larvae was unexpectedly high, with an average concentration of 2.20 mg/100 g. Additionally, enzymatic browning process in wasp larvae is closely associated with phenol oxidase (PO) activity. The simultaneous treatment of ascorbic acid at a concentration of 0.2% (w/v) and high hydrostatic pressure at 300 MPa significantly inhibited PO activity. Notably, the combined treatment exhibited a certain degree of efficacy in retaining the taste and texture of the larvae. To the best of our knowledge, this study pioneers the novel combined treatment aimed at mitigating browning in wasp larvae. Overall, our research reveals that wasp larvae boast a wealth of nutritional components, rendering them as a new resource food. Our research also provides an innovative approach for wasp processing.

Keywords

ascorbic acid treatment – edible insects – high hydrostatic pressure – phenol oxidase – umami

1 Introduction

The Food and Agriculture Organization of the United Nations (FAO) predicts a 70% increase in food demand by 2050, leading to food shortages, increased costs, and potentially affecting global stability (Diouf, 2009). Fortunately, edible insects can be proposed as an alternative resource due to their sustainable and environmental benefits (Sousa *et al.*, 2020; Tao and Li, 2018). Edible insects possess higher food conversion rates and may grow from waste streams from humans, animals, or plants, making them more sustainable to feed (Nowakowski *et al.*, 2020). Also, raising insects requires less land and water, and produces fewer greenhouse gases than other meat-producing animals (Hazarika and Kalita, 2023; Huis and Gasco, 2023; Raheem *et al.*, 2019). To date, edible insects have been consumed in nearly 80 countries across Asia, Africa, Central America, South America, and Oceania (Huis *et al.*, 2013; Niassy *et al.*, 2018), and have also gained more enthusiasm from Europeans (Jensen and Lieberoth, 2018). In addition, artificial breeding of edible insects is gaining popularity

(Maciel-Vergara *et al.*, 2021), leading to rapid growth in the related food industry (Ahn *et al.*, 2020; Schlüter *et al.*, 2017). To advance this promising food source, it is crucial to consider the nutritional value and processing methods of edible insects alongside breeding technology.

Edible insects can provide comparable nutritional value to traditional animal or plant foods (Fellows *et al.*, 2014; Raheem *et al.*, 2019). Edible insects are typically rich in essential amino acids, polyunsaturated fatty acids, and other micronutrients such as vitamin B, zinc, and iron (Fellows *et al.*, 2014; Raheem *et al.*, 2019). Nutrients in edible insects vary widely in species diversity, habitat, diet, and life cycle stage (Huis, 2003). Common edible insects include beetles (Coleoptera) (31%), caterpillars (Lepidoptera) (18%), bees, ants (Hymenoptera) (14%), and wasps (Hymenoptera: Vespidae) (Cerritos, 2009). Wasps, a diverse group of hymenopteran insects, boast approximately 70,000 species globally (Yoon *et al.*, 2020). Wasps display diverse the concentrations of protein, fat, carbohydrates, and micronutrients, manifesting significant variations across distinct species. For instance, the dry basis protein content of *Polistes instabilis* is 31%, while that of *Polybia sp.* can reach a much higher 81% (Ramos-Elorduy *et al.*, 1997). Research on *Polistes metricus* has revealed that larvae contain higher levels of protein and lipid compared to other stages, except for eggs (Judd *et al.*, 2010). In the province of Yunnan, China, the historical tradition of consuming wasp larvae spans over thousand years and enjoys widespread appreciation among the indigenous population. The three species of wasps, *Provespa barthelemyi*, *Vespa mandarinia* and *V. velutina*, are now commercially cultivated in Yunnan province to provide larvae for human consumption on a large scale. However, the nutritional constituents of these three artificially cultivated wasp larvae still remain undetermined.

As large-scale artificial cultivation, larval products are facing a primary challenge regarding browning during the processing and storing period. For better retain the product quality, finding promising ways to inhibit browning is of great importance. Numerous studies have shown that this browning about insect products has been primarily attributed to enzymatic browning caused by phenol oxidase (PO) (Wu, 2013). Efforts to inhibit PO activity have traditionally involved chemical and physical methods. Chemical methods usually use different reagents to inhibit PO activity. For example, PO activity in locust nymphs was inhibited by 4-hexylresorcinol, kojic acid, and quercetin (Rafiei *et al.*, 2017). The limitation of this method is that high doses of reagents are required, resulting in a change in taste and potential safety risks. Physical methods mainly include thermal and non-thermal treatment. While thermal treatment can destroy heat-sensitive nutrients, non-thermal treatment is increasingly used to inhibit PO activity, such as high hydrostatic pressure (HHP). In a previous study, HHP had the potential to prevent enzymatic browning in shrimp (Huang *et al.*, 2014). However, there are few studies using non-thermal treatment to address the browning problem of wasp larvae.

This study aimed at investigating the nutrient profiles of wasp larvae and browning inhibition treatments. Crude protein, amino acids, crude fat, fatty acids, nucleotides, vitamins, and minerals were quantified. Browning inhibition was evaluated after treatment with ascorbic acid (AA), high hydrostatic pressure (HHP), and their combination (AA-HHP), respectively.

2 Materials and methods

Samples

Three species of wasp larvae (*P. barthelemyi*, *V. mandarinia*, and *V. velutina*) were collected from nine apiaries located in Longling county Baoshan City in Yunnan Province, China in 2018. The mix larvae of different stages were carefully selected, packed in sealed bags and subsequently stored at −80 °C until further used.

Chemicals

Phosphate buffer, native mark unstained protein standards, and mark pre-stained protein standards were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Coomassie brilliant blue R-250, L-Dopa, acrylamide, N-N methyl bis acrylamide, Tris, and trifluoroacetic acid were purchased from Sigma (Sigma Chemical Co, Louis, MO, USA). Novex 4-12% tris-glycine gel, tris-glycine native sample buffer, tris-glycine sodium dodecyl sulfate (SDS) sample buffer, and tris-glycine native running buffer were purchased from Invitrogen (Invitrogen Ltd., Paisley, UK). Anhydrous acetonitrile, formic acid, and acetone were purchased from JT Baker (JT Baker, Phillipsburg, PA, USA). Pepsin, amylase, and papain were obtained from Promega (Promega Corporation, Madison, WI, USA). Ascorbic acid was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China).

Determination of nutrients and nucleotides

Nutritional analysis was conducted during the period spanning 2018 to 2019. Wasp larvae were removed from

–80 °C and immediately dried for 48 h in a freeze-dry system (LGJ-18S, Beijing Songyuan Huaxing Technology Develop Co., Ltd., Beijing, China). Then freeze-dried samples were ground to pass through a 200-mesh sieve (about 74 um). The dried particles were packed in sealed bags and stored at –18 °C until used. The Kjeldahl method was used to calculate crude protein content (Vit *et al.*, 2016). Amino acid content was determined using an automatic amino acid analyzer (L8900, Hitachi, Tokyo, Japan) according to the Chinese national standard (GB/T 18246-2019). Crude fat was determined using an automated fat extractor, Soxtec 8000 (Foss Analytical AB, Hilleroed, Denmark). Fatty acid compositions were determined by gas chromatograph-mass spectrometry (Agilent 7890A-5975C, Agilent Technologies, Santa Clara, CA, USA) (Dong *et al.*, 2015). According to previous research, we analyzed the contents of minerals (Cosmulescu *et al.*, 2015), vitamins (Nie *et al.*, 2021), and nucleotides (Bu *et al.*, 2019). Every measurement included three parallel samples.

Preparation of crude enzyme solution

According to the previous research (Shi *et al.*, 2017), the crude enzyme solution of wasp larvae was prepared with minor modifications. Wasp larvae were removed from –80 °C and immediately ground in ice-cold phosphate buffer at a ratio of 1:5 (g/mL). After crushing, the resulting samples were quickly transferred to a centrifugal tube and homogenized by an ultrasonic cell crusher (UP-250, Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China) for 2 min. After 30 min incubation at 4 °C, homogenates were centrifuged (Sorvall Biofuge Stratos, ThermoScientific, Hanau, Germany) at 12,000× g for 20 min at 4 °C. The supernatant was filtered with 16-layer sterile gauze, and the resulting filtrate was used as the crude enzyme solution. The crude enzyme solution was stored at –80 °C before subsequent use.

PO characterization

Samples were analyzed by gel electrophoresis and stained with Coomassie brilliant blue or L-Dopa for visualization. Coomassie brilliant blue staining can show varying electrophoretic protein profiles (Kunalan *et al.*, 2018). L-Dopa staining has traditionally been used to characterize insect POs (Cherqui *et al.*, 1996). This is because PO remains active under sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) conditions, like PO from *Blaberus discoidalis* (Blattodae: Blaberidae) (Durrant *et al.*, 1993). In other words, the black band appears in the PO position after L-Dopa staining.

Non-reducing SDS-PAGE analysis

Non-reducing SDS-PAGE was carried out as described by Laemmli (1970) with minor modifications. The crude enzyme solution was mixed with tris-glycine SDS sample buffer and loaded, without boiling, onto homemade 10% polyacrylamide gels. Mark pre-stained protein standards were used as molecular weight markers. The concentrated gel was run at 80 V, and the separation gel was run at 120 V constant pressure electrophoresis. After electrophoresis, the separation gel was cut into two gel slices (A and B). The proteins in gel slice A were stained with Coomassie brilliant blue, while the proteins in gel slice B were used for PO characterization. Gel slice B was equilibrated with phosphate buffer for 20 min and then stained with L-Dopa solution at 30 °C for 2 h.

Native-polyacrylamide gel electrophoresis (Native-PAGE) analysis

Native-PAGE was carried out according to the manufacturer's protocol (Invitrogen, Carlsbad, CA). The crude enzyme solution was prepared in a 2 × native sample buffer and loaded onto a precast Novex 4-12% tris-glycine gel. Native mark unstained protein standards were used as molecular weight markers. Native-PAGE was run with tris-glycine native running buffer at 80 V at 4 °C. After electrophoresis, the separation gel was cut into two gel slices (C and D). The proteins in gel slice C were stained with Coomassie brilliant blue, while the proteins in gel slice D were used for PO characterization as described above.

PO sequence identification using LC-MS/MS

To identify the PO sequence, the PO corresponding band was cut from gel slice C (see Native-PAGE analysis session for details) for in-gel digestion and mass spectrometry (Qiao *et al.*, 2018). Briefly, the band was washed and cut into small pieces. These pieces were then disulfide reduced with 25 mM dithiothreitol and alkylated with 50 mM iodoacetamide. Overnight digestion was performed with pepsin at 37 °C. The peptides were then extracted for 30 min using an aqueous 50% acetonitrile solution containing 0.1% trifluoroacetic acid. The peptide extract was centrifuged in a SpeedVac to reduce volume.

Peptide analysis was performed using an Orbitrap Fusion mass spectrometer coupled to an Easy-nLC 1000 system (Thermo-Fisher Scientific, Waltham, MA,USA). Peptide solution was eluted with a 60 min gradient on a Thermo Scientific Acclaim PepMap C18 column (100 μm × 2 cm, particle size 3 μm). Mobile phase A was 0.1% formic acid in water, and mobile phase B was

0.1% formic acid in acetonitrile. The total flow rate was set at 300 nL/min. The mass spectrometer was operated using data-dependent acquisition (DDA) by Xcalibur software. There was a single full-scan mass spectrum in Orbitrap followed by top-speed MS/MS scans in the ion trap. MS/MS spectrums were searched against the NCBI database for Vespidae using Proteome Discoverer (version 1.4, Thermo-Fisher Scientific, Waltham, MA, USA).

Assay for PO activity

PO activity in wasp larvae was determined based on previous research with some modifications (Nirmal and Benjakul, 2009). The assay system consisted of 200 μL crude enzyme solution, 1 mL of 15 mM L-Dopa in deionized water, and 2 mL of 50 mM phosphate buffer. PO activity was determined for 3 min at 45 °C by monitoring dopachrome formation at 475 nm using a UV-vis spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan). A total of three parallel experiments were conducted in each group. PO activity in U/L is the change in absorption per minute (ΔA/min).

Browning inhibition treatments

Ascorbic acid (AA) treatment

The effect of AA on browning in wasp larvae was evaluated by PO activity. AA was dissolved in distilled water to various concentrations (0%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, w/v, respectively). Wasp larvae were placed in an AA solution and incubated at room temperature for 4 h. Following this, crude wasp larvae enzyme solution was prepared, and the PO activity of the enzyme solution was determined as described above. Relative PO activity (%) = $A_X/A_0 \times 100$, where A_X is the PO activity in the presence of AA, and A_0 is the PO activity of control (without AA). IC_{50} was measured using Probit Analysis on SPSS Statistics version 17.0 (SPSS Inc., Chicago, IL, USA).

High hydrostatic pressure (HHP) treatment

The HHP treatment was performed by HPP.W1 with a high hydrostatic pressure processor (Huataisenmiao Biology Engineering Technology Co., Ltd., Tianjin, China). Briefly, wasp larvae were packed in a polyethylene (PE) bag (7 cm × 12 cm) with the appropriate amount of distilled water and pressurized at 0, 100, 200, 300, 400, or 500 MPa for 5 or 15 min. After treatment, residual PO activity was determined as described above.

Combination of ascorbic acid and high hydrostatic pressure

The combined treatment was carried out with a high hydrostatic pressure unit. Wasp larvae were packed in a polyethylene (PE) bag (7 cm × 12 cm) with the appropriate amount of 0.2% (w/v) AA and pressurized at 300 MPa for 0, 5, 10, 15, 20, or 25 min. After treatment, residual PO activity was determined as described above.

Statistical analysis

Results were expressed as mean ± standard deviation (SD) from triplicate measurements. ANOVA followed by Duncan's Multiple-Range Test was used to compare significant differences at $P < 0.05$ among treatment means. SPSS statistics software (version 17.0; SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

3 Results

Nutrient profiles

Crude protein and amino acids

Table 1 presents a comprehensive assessment of crude protein and amino acid contents in three species of wasp larvae on a dry weight basis. The predominant constituent in wasp larvae was protein, constituting more than 50% of the dry weight. Crude protein contents of the three wasp larvae were not significantly different ($P > 0.05$), ranging from 516.28 to 557.57 g/kg. In addition, these wasp larvae were rich in various amino acids (AA), and all contained eight essential amino acids (EAA). These essential amino acids (EAA) occupied about one-third of total amino acids (TAA), and the EAA/TAA ratios of the three larvae varied from 0.36 to 0.37. It is worthwhile to note that lysine, the primary limiting essential amino acid of cereal grains, was found at high levels from 30.10 to 33.47 g/kg in wasp larvae. Another notable finding was that glutamic acid and aspartic acid were the most abundant amino acids in wasp larvae. These two amino acids, closely related to umami taste, accounted for over a quarter of TAA. Sweet amino acids constituted nearly one-fifth of TAA, including threonine, serine, glycine, and alanine. Moreover, the content of each amino acid in *V. mandarinia* was higher than that of *V. velutina*, especially glutamic acid.

Crude fat and fatty acids

Table 2 illustrates variations in the contents of crude fats and fatty acids (FAs) among wasp larvae on a dry weight basis. As can be seen, crude fat accounted for over one-

TABLE 1 Protein contents and amino acid compositions of wasp larvae (g/kg)*

Contents (g/kg)	*Provespa barthelemyi*	*Vespa mandarinia*	*V. velutina*
Crude protein	535.91 ± 29.49^a	557.57 ± 43.44^a	516.28 ± 22.26^a
Essential amino acids			
Isoleucine (Ile)	23.43 ± 0.35^b	26.51 ± 0.97^a	22.40 ± 1.30^b
Leucine (Leu)	40.40 ± 0.36^b	45.79 ± 0.67^a	38.70 ± 1.60^b
Lysine (Lys)	31.08 ± 0.37^{ab}	33.47 ± 1.12^a	30.10 ± 1.90^b
Methionine (Met)	8.05 ± 0.03^a	8.13 ± 0.23^a	8.03 ± 0.35^a
Phenylalanine (Phe)	19.71 ± 0.27^a	20.42 ± 0.69^a	18.97 ± 1.15^a
Threonine (Thr)	19.94 ± 0.09^b	23.65 ± 2.79^a	19.10 ± 1.00^b
Tryptophan (Trp)	4.90 ± 0.07^b	5.81 ± 0.16^a	3.50 ± 0.10^c
Valine (Val)	28.86 ± 0.37^b	33.12 ± 0.86^a	26.27 ± 1.55^c
Non-essential amino acids			
Alanine (Ala)	24.19 ± 0.19^b	29.03 ± 0.66^a	21.49 ± 0.55^c
Arginine (Arg)	22.27 ± 0.34^b	24.44 ± 0.86^a	20.50 ± 1.11^c
Aspartic acid (Asp)	47.25 ± 1.41^a	49.00 ± 1.30^a	44.50 ± 2.60^a
Cysteine (Cys)	3.49 ± 0.03^b	3.68 ± 0.05^a	3.31 ± 0.08^c
Glutamic acid (Glu)	94.30 ± 1.51^b	103.85 ± 2.12^a	83.13 ± 5.25^c
Glycine (Gly)	30.03 ± 0.21^b	35.42 ± 0.77^a	25.33 ± 1.05^c
Histidine (His)	15.66 ± 0.11^a	16.42 ± 0.50^a	13.60 ± 0.80^b
Proline (Pro)	24.87 ± 0.67^b	28.30 ± 0.29^a	21.00 ± 1.00^c
Serine (Ser)	20.37 ± 0.08^a	21.39 ± 0.43^a	19.77 ± 1.15^a
Tyrosine (Tyr)	33.38 ± 0.61^a	34.69 ± 1.27^a	31.37 ± 2.05^a
Total amino acids	492.18b	543.12a	451.07c

Each value is shown as the mean of three determinations ± the standard deviation; different letters indicate significant differences ($P < 0.05$); *based on dry matter.

fifth of wasp larvae, with an average of 219.83 g/kg. There was a significant difference in crude fat content between *V. mandarinia* larvae and *V. velutina* larvae ($P < 0.05$). On the other hand, wasp larvae species varied considerably in fatty acid compositions. These samples contained many FAs, ranging from 17 types in *P. barthelemyi* larvae to 21 types in *V. velutina* larvae. Ten types of saturated fatty acids (SFAs), five types of monounsaturated fatty acids (MUFAs), and seven types of polyunsaturated fatty acids (PUFAs) were identified in larvae samples. The total contents of FAs in wasp larvae ranged from 201.97 g/kg in *V. mandarinia* larvae to 224.21 g/kg in *V. velutina* larvae. In three wasp larvae, unsaturated fatty acids (UFAs) were higher than SFAs, and PUFAs/SFAs ratios were above 0.3. Notably, all larvae samples showed high levels of n-6/n-3 PUFA with an average of 0.89, and the highest was 0.99 for *V. mandarinia* larvae.

Vitamin, mineral, and nucleotide contents

Table 3 reveals a wide variation in vitamin, mineral, and nucleotide contents in three wasp larvae on a dry weight basis. All larvae samples showed high levels of iron and zinc with averages of 10.56 mg/100 g and 8.36 mg/100 g, respectively, and the lowest of 10.11 mg/100 g and 7.17 mg/100 g, respectively. It is worth noting the high contents of vitamin B_2 and vitamin E, especially vitamin B_2 with an average of 2.20 mg/100 g. Another notable result was that wasp larvae species varied significantly in nucleotide contents. As umami nucleotide, the contents of inosine monophosphate (IMP) were higher than those of guanosine monophosphate (GMP) in all samples, reaching a maximum level of 66.88 mg/100 g in *V. velutina* larvae.

Identification of PO in wasp larvae

Electrophoretic analysis

Gel slices A and B of non-reducing SDS-PAGE stained with Coomassie brilliant blue and L-Dopa are presented in Figure 1 (A and B). As can be seen, the electrophoretic patterns of the three larvae looked extremely similar with some clear bands at about 10, 70, 100, 130, 180, and 260 kDa (Figure 1A). After L-Dopa staining, black bands

TABLE 2 Crude fat contents and fatty acid compositions of wasp larvae (g/kg)*

Contents (g/kg)	*Provespa barthelemyi*	*Vespa mandarinia*	*V. velutina*
Crude fat	219.77 ± 6.49^{ab}	205.43 ± 11.46^{b}	234.30 ± 6.21^{a}
Saturated fatty acids			
Capric acid (10:0)	0.08 ± 0.00^{a}	0.07 ± 0.00^{b}	0.06 ± 0.01^{c}
Lauric acid (12:0)	3.80 ± 0.01^{b}	2.91 ± 0.01^{c}	4.36 ± 0.02^{a}
Tridecanoic acid (13:0)	ND	0.16 ± 0.00	ND
Myristic acid (14:0)	12.85 ± 0.07^{b}	10.27 ± 0.06^{c}	14.11 ± 0.05^{a}
Pentadecanoic acid (15:0)	0.07 ± 0.00^{c}	0.10 ± 0.00^{a}	0.08 ± 0.00^{b}
Palmitic acid (16:0)	56.07 ± 0.33b	59.79 ± 0.52^{a}	53.95 ± 0.04^{c}
Heptadecanoic acid (17:0)	0.55 ± 0.04^{b}	0.35 ± 0.00^{c}	0.73 ± 0.02^{a}
Stearic acid (18:0)	18.29 ± 0.4^{b}	12.27 ± 0.09^{c}	19.45 ± 0.13^{a}
Arachidic acid (20:0)	1.28 ± 0.02^{b}	1.15 ± 0.01^{c}	1.36 ± 0.01^{a}
Behenic acid (22:0)	ND	ND	0.54 ± 0.03
Monounsaturated fatty acids			
Myristoleic acid (14:1)	0.55 ± 0.00^{b}	0.05 ± 0.00^{c}	0.59 ± 0.00^{a}
Palmitoleic acid (16:1)	5.45 ± 0.04^{b}	3.28 ± 0.02^{c}	6.59 ± 0.03^{a}
Heptadecenoic acid (17:1)	0.26 ± 0.01^{b}	0.21 ± 0.00^{c}	0.27 ± 0.00^{a}
Oleic acid (18:1)	88.36 ± 0.45^{b}	82.78 ± 0.42^{c}	91.47 ± 0.29^{a}
Eicosenoic acid (20:1)	0.17 ± 0.01^{b}	0.14 ± 0.00^{c}	0.22 ± 0.01^{a}
Polyunsaturated fatty acids			
Linoleic acid (18:2 n-6)	12.97 ± 0.02^{b}	13.52 ± 0.09^{a}	12.58 ± 0.08^{c}
α-Linolenic acid (18:3 n-3)	15.39 ± 0.09^{b}	13.93 ± 0.11^{c}	16.07 ± 0.06^{a}
γ-Linolenic acid (18:3 n-6)	ND	ND	0.07 ± 0.01
Eicosadienoic acid (20:2)	ND	ND	0.07 ± 0.01
Eicosatrienoic acid (20:3 n-3)	ND	ND	0.05 ± 0.00
Arachidonic acid (20:4 n-6)	0.80 ± 0.00^{b}	0.66 ± 0.00^{c}	0.87 ± 0.00^{a}
Eicosapentaenoic acid (20:5 n-3)	0.56 ± 0.02^{b}	0.33 ± 0.00^{c}	0.72 ± 0.00^{a}
Total fatty acids	217.50^{b}	201.97^{c}	224.21^{a}

ND = not detected. Each value is the mean of three determinations ± the standard deviation; different letters indicate significant differences ($P < 0.05$); *based on dry matter.

in three lanes appeared at similar locations (Figure 1B). A similar PO likely exists in the three larvae.

Native-PAGE analysis was further employed to investigate protein polymerization states, as proteins typically retain their native structure and activity under this condition. As can be seen from gel slices C and D of native electrophoresis (Figure 1 C and D), the molecular weights of PO from *P. barthelemyi*, *V. mandarinia*, and *V. velutina* larvae were detected at approximately 680, 809, and 763 kDa, respectively. Therefore, the PO in the three larvae was inferred to be a polymer consisting of monomers. This difference observed on Native-PAGE may result from varying degrees of polymerization. For example, the previous research showed that PO tends to aggregate with each other or with some macromolecules through hydrophobic interaction (Sugumaran *et al.*, 2000; Zufelato *et al.*, 2005).

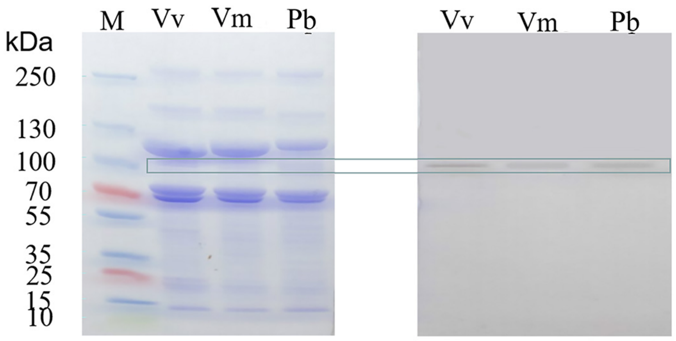
PO sequence identification

The corresponding PO bands were analyzed by LC-MS/MS. The data shown in Table 4 illustrated that three larvae POs were identified as phenoloxidase2-like in *Polistes canadensis* (Hymenoptera: Vespidae) with a molecular weight of 80.1 kDa and an isoelectric point of 6.64. Inputting this PO protein sequence to AlphaFold2, it was predicted that PO protein contained 31 α-helix structures (67%) and 15 β-sheet structures (33%) (Supplementary Figure S1). Overall, the POs in wasp larvae consisted of some monomers, for one monomer with the molecular weight of 80.1 kDa. Thus, we suppose that the 680 kDa, 763 kDa, and 809 kDa forms may result from the aggregation of eight, nine, and ten 80.1 kDa monomers.

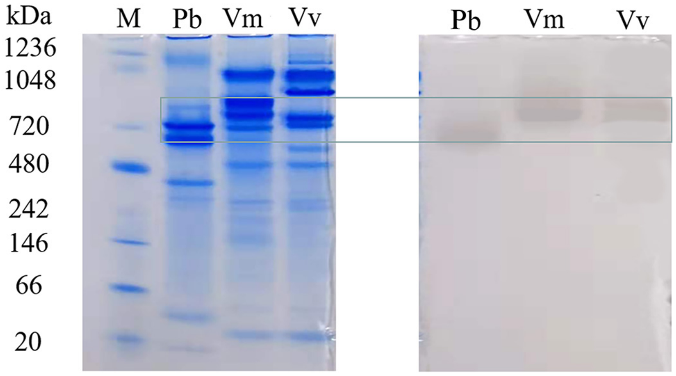
TABLE 3 Vitamin, mineral, and nucleotide contents of wasp larvae (mg/100 g)*

Contents (mg/100 g)	*Provespa barthelemyi*	*Vespa mandarinia*	*V. velutina*
Minerals			
Iron	10.63 ± 1.34^{a}	10.93 ± 0.22^{a}	10.11 ± 0.49^{a}
Zinc	8.37 ± 0.32^{b}	9.55 ± 0.10^{a}	7.17 ± 0.41^{c}
Selenium	0.02 ± 0.00^{b}	0.02 ± 0.00^{a}	0.01 ± 0.00^{c}
Vitamins			
Vitamin E	2.46 ± 0.02^{ab}	2.38 ± 0.08^{b}	2.53 ± 0.02^{a}
Vitamin B$_1$	0.17 ± 0.00^{b}	0.13 ± 0.01^{c}	0.21 ± 0.00^{a}
Vitamin B$_2$	2.24 ± 0.02^{b}	2.08 ± 0.02^{c}	2.29 ± 0.03^{a}
Nucleotides			
Adenosine monophosphate (AMP)	5.11 ± 0.07^{b}	6.50 ± 0.18^{a}	3.03 ± 0.12^{c}
Cytidine monophosphate (CMP)	3.96 ± 0.07^{b}	5.85 ± 0.26^{a}	2.14 ± 0.20^{c}
Guanosine monophosphate (GMP)	11.24 ± 0.05^{a}	ND	9.11 ± 0.01^{b}
Inosine monophosphate (IMP)	43.77 ± 0.28^{b}	7.75 ± 0.37^{c}	66.88 ± 0.59^{a}
Uridine monophosphate (UMP)	57.72 ± 0.30^{b}	ND	71.11 ± 0.28^{a}

Each value is the mean of three determinations ± the standard deviation; different letters indicate significant differences ($P < 0.05$); *based on dry matter.



SDS-PAGE CBBS (A) SDS-PAGE LDS (B)



Native-PAGE CBBS (C) Native-PAGE LDS (D)

FIGURE 1 Non-reducing SDS-PAGE analysis (A and B) and Native-PAGE analysis (C and D) of wasp larvae. (A and C) stained with CBBS (Coomassie brilliant blue); (B and D) stained with LDS (L-Dopa); Lane M = protein molecular weight marker; lane Pb = *Provespa barthelemyi*; lane Vm = *Vespa mandarinia*; lane Vv = *V. velutina*.

TABLE 4 Identification of protein bands on Native-PAGE using LC-MS/MS*

Fractions	Accession NO.	Coverage (%)	Peptides	MW (kDa) /PI	Protein name
Pb	XP_014613621.1	14.10	6	80.1/6.64	phenoloxidase2-like OS = Polistescanadensis
Vm	XP_014613621.1	14.24	6	80.1/6.64	phenoloxidase2-like OS = Polistescanadensis
Vv	XP_014613621.1	7.63	7	80.1/6.64	phenoloxidase2-like OS = Polistescanadensis

Mw = molecular weight; pI = isoelectric point; *Thermo Orbitrap Fusion mass spectrometer; Pb = *Provespa barthelemyi*; Vm = *Vespa mandarinia*; Vv = *V. velutina*.

Inhibitory effects of different treatments on PO

Effects of ascorbic acid (AA) on PO in wasp larvae

The inhibitory effects of AA on PO activity in three wasp larvae were determined (Figure 2A). As can be seen, AA exhibited good potency in inhibiting PO activity. Residual PO activities decreased with increasing AA concentrations and were almost completely lost when treated with 0.5% (w/v) AA. However, high concentrations of AA also affect the pH of wasp larvae. When the addition of AA exceeded 0.3% (w/v), the pH value dropped below 6, leading to the overly sour taste of wasp products. For *P. barthelemyi*, *V. mandarinia*, and *V. velutina* larvae, the half maximal inhibitory concentration (IC_{50}) values of AA on PO activity were estimated to be 0.216%, 0.244% and 0.217% (w/v), respectively. Therefore, considering the taste quality, 0.2% (w/v) AA was selected in the following combination study.

Effects of high hydrostatic pressure (HHP) on PO in wasp larvae

The effects of HHP on PO activity in wasp larvae treated for 5 or 15 min are shown in Figure 2 (B and C). As can be seen, it could activate POs at pressures below 200 MPa, while significantly inhibiting POs at pressures above 300 MPa. PO activities decreased with increasing pressure levels above 300 MPa and were almost wholly lost at 500 MPa. Of the three wasp larvae, the PO of *V. mandarinia* was the least likely to be inactivated. When treated for 5 min, pressures above 400 MPa effectively inhibited PO activities (Figure 2B). When treated for 15 min, PO activities can be effectively suppressed at pressures above 300 MPa (Figure 2C). If the pressure exceeds 300 MPa, the texture of the product will probably be affected. Pressure 300 MPa was suitable for the following combination study.

Effects of the combination of ascorbic acid and high hydrostatic pressure (AA-HHP) on PO in wasp larvae

Figure 2D reveals the effects of AA-HHP on the PO activity of wasp larvae. The combination method (0.2% (w/v) AA, pressure 300 MPa) had a better inhibitory effect on PO activity than a single treatment. PO activities in three larvae decreased with the growth of AA-HHP treatment time. When treated for 5 min, PO activities can be reduced to about one-third of the original activities. When treated for 10 min, PO activity can be reduced to less than 20% of the original activity for *P. barthelemyi* and *V. velutina* larvae, while 20 min for *V. mandarinia* larvae.

4 Discussion

The "Greater Food" concept has recently gained popularity to meet the growing demand for food by an expanding population (Liu, 2022). Historically, most protein-rich foods were obtained from conventional agriculture, livestock, and poultry resources, which are potentially unfriendly to the environment (Ortega-Urquieta *et al.*, 2022; Steinfeld *et al.*, 2006). With the improvement in living standards, various eating habits have emerged. Currently, "Greater Food" refers to the expansion of food sources and harvesting methods (Zhou *et al.*, 2022), promoting sustainable agriculture and ecological conservation in a more balanced manner. This approach advocates for exploring alternative biological resources, such as edible insects.

As a natural, renewable resource, edible insects may have occurred in the human diet for tens of thousands of years. Until today, the habit of eating insects has been maintained in many areas. For instance, in Thailand, edible insects such as beetles, crickets, locusts, and wasps are consumed by rural residents and served as premium meals or snacks for visitors (Yen *et al.*, 2013;

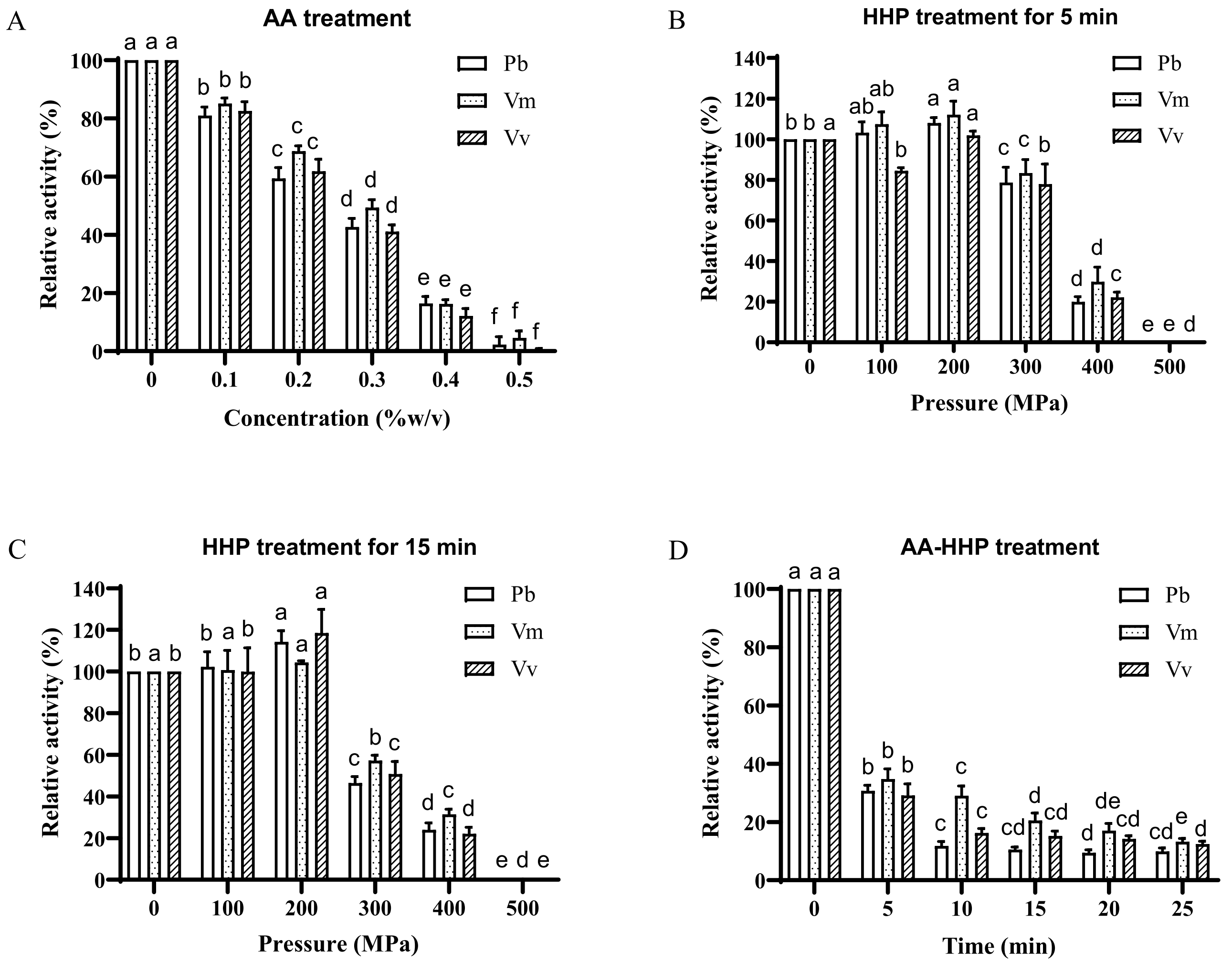


FIGURE 2 Effects of ascorbic acid, high hydrostatic pressure, and their combination treatment on phenol oxidase activities of wasp larvae. Values with different letters in the same column are significantly different ($P < 0.05$) from each other. Pb = *Provespa barthelemyi*; Vm = *Vespa mandarinia*; Vv = *V. velutina*; (A) treated with ascorbic acid (AA); (B) treated with high hydrostatic pressure (HHP) for 5 min; (C) treated with high hydrostatic pressure (HHP) for 15 min; (D) treated with ascorbic acid-high hydrostatic pressure (AA-HHP).

Yhoung-Aree *et al.*, 1997). In China, historical records from the Tang Dynasty provide detailed accounts of the collection and culinary practices associated with wasp larvae (Zou, 1981). In Mexico, there exist varieties of readily wasp larvae, commonly consumed both raw and roasted (Wen, 1998). In Japan, wasp larvae are preferred and treasured as an autumn delicacy, and canned wasp larvae are commercially available in local markets (Nonaka, 2010). Wasp larvae are universally enjoyed probably because of their unique tastes and high nutritional values. As is well known, the taste characteristics of food are mainly associated with specific taste components. It is worthwhile to note that the principal taste of wasp larvae involves umami and sweetness. Our research shows that umami taste in wasp larvae may be due to the high levels of two amino acids (Glu and Asp) and two nucleotides (IMP and GMP). Glu and Asp were found to be the most abundant amino acids (Table 1); particularly, Glu content in each sample was about three times higher than in beef (Yang *et al.*, 2022). Furthermore, IMP levels were much higher than GMP levels in all wasp larvae (Table 3). It may be because IMP is primarily present in animal-derived foods, while GMP mainly exists in plant-derived foods. In addition, IMP in *V. velutina* larvae was about 12 times more abundant than in beef (Yang, 2012). The role of IMP in wasp larvae appears to be consistent with that of the research reporting that IMP is the primary substance of umami taste in animal-derived foods such as Shanghai smoked fish and chicken soup (Feng *et al.*, 2016; Xue *et al.*, 2019). Moreover, four amino acids (Thr, Ser, Gly, and Ala) may play a particular role in the sweet taste of wasp larvae, accounting for nearly one-fifth of total amino acids (Table 1). Overall, these findings provide important insights into the consumption of edible insects due to their umami and sweetness.

Our results demonstrate that wasp larvae possess a high nutritional value. Firstly, all three larvae samples had high protein contents and nutritionally balanced amino acid compositions. In our study, the protein content in wasp larvae exceeded 50% (Table 1), further supporting the previous report showing that proteins occurring in edible insects typically range from 7% to 91% (Huis, 2016). Furthermore, the mean protein content of three larvae samples slightly exceeded the protein content of *V. velutina nigrithorax* larvae from South Korea (Jeong *et al.*, 2020). The average protein content in the samples was about 1.4 times higher than in soybeans (Feng *et al.*, 2018) and 2.2 times higher than in beef (Wang *et al.*, 2019a). Surprisingly, all wasp larvae samples contained EAA/TAA ratios similar to those recommended by FAO/WHO (Wang *et al.*, 2019b). In addition, lysine levels in three wasp larvae were significantly higher than in *V. velutina nigrithorax* larvae (Jeong *et al.*, 2020), beef (Xie *et al.*, 2019) or soybeans (FRIAS *et al.*, 2007). Secondly, virtually all samples of wasp larvae were characterized by an appropriate amount of crude fat and a wide variety of fatty acids (FAs). These samples contained approximately 22% crude fat (Table 2), comparable to the crude fat content found in *V. mandarinia* larvae from Korea (Jeong *et al.*, 2020). Notably, this crude fat content was found to be lower than that of pork belly but higher than that of pork buttock (Lin *et al.*, 2014). In addition, compared to other meats such as beef, much more unsaturated fatty acids (UFAs) were found in larvae samples (Scollan, 2003). UFAs, especially PUFAs, can play a crucial role in the prevention and control of human diseases. The composition of PUFAs in cell membranes varies considerably with diet; therefore, balancing the n-6/n-3 PUFAs ratio is crucial (Gago-Dominguez *et al.*, 2003). Today, the unbalanced diet of humans has seriously deviated from the recommended level, n-6/n-3 PUFAs of 4-6:1 (Kris-Etherton *et al.*, 2000). Our results reveal that n-6/n-3 PUFAs in wasp larvae met the recommendation and were better than those reported in beef (Scollan, 2003). Thus, wasp larvae may help regulate the balance of n-6/n-3 PUFAs in human diets. Thirdly, wasp larvae can provide various minerals and vitamins needed for the physiological development of individuals. In our study, iron and zinc reached an average of 10.56 and 8.36 mg/100 g in three wasp larvae samples, respectively (Table 3). The mean iron content in larvae samples is consistent with previously reported iron content in *V. velutina* larvae from China, slightly exceeding iron content in *V. velutina* larvae from Korea (Ghosh *et al.*, 2021). The mean zinc content of larvae samples is 1.4 times higher than that of *V. velutina* larvae from Korea (Ghosh *et al.*, 2021). These two values are not only higher than those of common plant foods such as black cornmeal, green peas, and kidney beans (Castro-Alba *et al.*, 2019), but also much higher than those of familiar animal foods such as pork, beef, and chicken (Bohrer, 2017). Wasp larvae may have the potential to alleviate iron or zinc deficiency. Most notably, vitamin B_2 occurred at high levels in all samples of wasp larvae (Table 3), and their contents were about ten times greater than those of meat products (Shen, 1988). It appears that wasp larvae can help prevent riboflavin deficiency, such as rosacea keratitis and scrotal dermatitis. Overall, wasp larvae are abundant-nutrient food.

Wasp larvae possess a wide range of nutrients, but their application may be restricted due to enzyme browning. Our findings reveal that enzyme browning in three larvae samples is most probably associated with POs, consisting of some monomers, for one monomer with the molecular weight of 80.1 kDa (Figure 1 and Table 4). A similar PO was previously reported to exist in silkworms (Ma *et al.*, 2009). Furthermore, in the native state, PO appeared to exist in diverse polymerized forms in wasp larvae. Thus, inhibiting PO activity may slow down and even block enzymatic browning. Prior work has documented numerous strategies for mitigating browning in other meat products, encompassing both chemical and physical processes. However, there exists a need for a robust approach to inhibit the enzymatic browning of wasp larvae. In this study, we used three different treatments to inhibit PO activity. When AA alone was used as the inhibitor for PO activity, the minimum relative activity could be obtained at 0.5% (w/v) (Figure 6A). Although AA may almost completely deactivate PO activity, it has a detrimental effect on the taste of wasp larvae. Similar to shrimp (Huang *et al.*, 2014), high hydrostatic pressure (HHP) can slightly activate PO below 200 MPa (Figure 6 B,C). This may be because lower pressure can dissociate PO and expose more active sites. When the pressure exceeded 300 MPa, PO activity became weaker with higher pressure (Figure 6 B,C). We hypothesize that the tertiary structure of the protein may be disrupted by increased pressure, and further alteration of amino acids in the active center ultimately changes PO activity. In addition, the pressure of 500 MPa can cause the complete inactivation of PO (Figure 6 B,C). However, higher pressure may cause a series of quality changes in meat products. For instance, pressures above 400 or 500 MPa tend to adversely affect the texture of prawns, fish or beef (Li *et al.*, 2019; Ma and Ledward, 2004; Matser *et al.*, 2000;

Yoshioka and Yamamoto, 1998). Consequently, pressures below 500 MPa seem to be more conducive for ensuring the texture and flavor attributes of food. In particular, a combination method (AA-HHP) had a better inhibitory effect on PO activity than a single treatment. For example, PO activity in *P. barthelemyi* or *V. velutina* larvae can be passivated after AA-HHP treatment for only 10 min (Figure 6D). HHP treatment seems to expose more active sites of PO protein, thereby facilitating the binding of ascorbic acid to the active sites, and subsequently leading to the enzyme inactivation. Thus, the synergistic effects of high pressure and ascorbic acid lead to the rapid deactivation of the PO enzyme. Most strikingly, this combination method requires only 0.2% (w/v) AA and a pressure of 300 MPa. Using this combination method, the taste and texture of wasp larvae can be preserved as much as possible. To our knowledge, the combined treatment of ascorbic acid and high hydrostatic pressure appears reliable and innovative to inhibit browning in wasp larvae.

5 Conclusion

Our study has unveiled that the consumption of wasp larvae can be suggested as being a favorable source of nourishment, given their umami flavor, sweetness, elevated protein proportion, and copious nutritional value. The synergistic effect of utilizing high hydrostatic pressure in conjunction with ascorbic acid not only serves to inhibit enzymatic browning, but also ensures an optimal retention of the palatable taste and texture inherent within wasp larvae. Future research will confirm whether this technique has potential application in other edible insects.

Supplementary material

Supplementary material is available online at:
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Author contributions

K. Wang: conceptualization, methodology, investigation, formal analysis, writing – original draft, visualization. H. Zhu and X. Chen: investigation, data curation, visualization. J. Qiao: investigation, data curation. G. Huang: investigation, resources, project administration. E. Haubruge: conceptualization. J. Dong: conceptualization, methodology, data curation. H. Zhang: conceptualization, validation, resources, writing – review and editing, funding acquisition.

Conflict of interest

The authors have no conflict of interest to declare.

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