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Nutritional composition of *Polyrhachis vicina* Roger (Edible Chinese black ant)

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Abstract

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Edible black ant (*Polyrhachis vicina* Roger) is a traditional edible insect species in China. It has been used as a functional ingredient in various tonics or health foods. This study determined the nutritional composition of the black ant, which included minerals, amino acids, superoxide dismutase (SOD), Vitamin E, and total acid. Supercritical CO_2 fluid extraction was used to extract the organic compounds. The compounds were identified and quantified by GC-MS. Results showed that the ant powder contained 77000 IU/100g of SOD, 56.6g/100g protein, 9.0g/100g fat, 13.2g/100g volatile oil, 6.0g/100g moisture, 1.6g/100g total acid and 6.3g/100g ash. There were 18 amino acids, of which, glutamic acid, glycine, aspartic acid, alanine, leucine, proline and tyrosine were predominant. Among the 16 minerals, K, Ca, P, Mg, Fe, Mn and Zn were predominant. More than 20 organic components were identified, the main ones were 9-octadecenoic acid, ethyl oleate, cholesterol and n-hexadecanoic acid. Six of the compounds found, i.e. hexadecanoic acid, ethyl ester, linoleic acid, ethyl oleate, oleic acid and cholesta-3, 5-diene, have not been reported previously. The results indicate that *P. vicina* Roger is rich in nutrients and is a potential ingredient for health food.

Key words : nutritional composition, *Polyrhachis vicina* Roger, Chinese black ant

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In ancient China, edible ants were called Xuanju and were widely used in Chinese medicines. During Zhou dynasty (11th century BC-256 BC), larvae of the ants were tribute food to be eaten by the emperors and the nobles (Li, 1999; Tang *et al*., 1995). Edible ants are still used as an ordinary food in some ethnic districts of Yunang, Guangxi and Guizhou in China (Wu, 1994). People in some ethnic districts of Mexico and Africa also have the habit of eating edible ants (Elorduy *et al*., 1997; DeFoliart, 1999).

Chinese black ant, *Polyrhachis vicina* Roger belongs to Formicidae, Hymenoptera, Insecta in zootaxy, and is widely distributed in subtropical southeast China, India, Malaysia, Srilanka and Bangladesh (Tang *et al*., 1995). It has been used as a nutritional ingredient and processed into various tonics or health foods. Its products available in Chinese market include powder products, drinks such as wines and capsules (Zhon, 1996; Shen and Ren, 1999). More than 30 ant-containing health products have been approved by the State Food and Drug Administration or State Health Ministry of China since 1996. Some ant products have been exported to Japan, South Korea, Thailand and other Southeast Asian countries. Ants are rich in nutrients (Cai *et al*., 1993; Li *et al*., 1995), and have several healthcare functions, e.g. regulating immune system, relaxing fatigue and anti-aging (Tian and Zhang, 2002). However, their nutritional compositions, especially functional characteristics, have not been sufficiently elucidated. The purpose of this study was to determine some nutritional composition of edible Chinese black ants.

Materials and Methods

Ant samples

The adult colonies of the edible Chinese black ant, *P. vicina* Roger were captured from their nests in the clump of bushes on hillsides in Linan, Tonglu and Hangzhou suburbs, Zhejiang Province, China. They were oven dried at 60-70ºC for a day, and stored at room temperature (18-25ºC). The dried ant samples were mechanically milled into powder with flat-hammer grinding mill and sifted through a 60-mesh screen. The powder was stored in sealed aluminum foil bag at 4ºC before analysis.

Analytical methods

The analysis was performed using the standard method of the People's Republic of China National Standard (PRCNS, GB). Total protein was determined using Kjeldahl method (GB 5009.5-1996). Moisture was determined using direct oven method at 130ºC for 1 h (GB 5009.3-1996). For total fat, Soxhlet extraction with ether was used (GB 5009.6-1996). The ash was determined gravimetrically (GB 5009.4-1996).

pH and total titratable acidity

The ant powder (1.0-2.0 g) was diluted with 100 mL distilled water. After 2 h, the solution was filtered with a filter paper. The filtered solution (20.0 mL) was diluted with 100 mL distilled water and mixed with a magnetic stirrer. The pH of the diluted solution was measured using a Metter Toledo 320 pH meter at 20ºC. Total titratable acidity was determined potentiometrically using 0.05 mol NaOH to the titration end point at pH 8.2 and expressed as g formic acid per 100g.

Amino acid, vitamin and superoxide dismutase determination

Amino acid was analyzed using automatic amino acid analyzer (Hitachi 830-50, Japan) according to the method of Li *et al*. (1994). The ant powder sample (1.0 g) was diluted with 0.02 M HCl. After 24 h, the solution was added with 5 mL of 4% sulphonic salicylic acid and kept for 1 h, then centrifuged for 10 min at 1000 rpm. A 50 µL sample of the supernatant was used in the determination of free amino acids with the automatic analyzer equipped with a 4×150 mm tubar column, at a constant linear velocity of 0.45 m/min for a total of 72 min. Another ant powder sample (0.5 g) was diluted with 5mL of 6M HCl and 3 drops of phenol freshly prepared. The solution was hydrolyzed for about 1 h at 110ºC. After the hydrolysis, the solution was filtered and diluted with 0.02 mol HCl. A 50 µL sample of the solution was used to determine the amino acids of the

hydrolyzed protein on the automatic analyzer at the same conditions as above.

Amino acid scores (mg of essential amino acid in 1.0 g of the protein/mg of the same amino acid in 1.0 g of the reference pattern of 9 essential amino acids plus tyrosine and cystine) were calculated by using FAO/WHO (1973) suggested pattern of amino acid requirements (Table 3). The lowest amino acid ratio was termed amino acid score (AAS).

Vitamin E (Tocopherols) was analyzed using HPLC according to the method of GB 12388-1996. Superoxide dismutase (SOD) was analyzed using chemical luminescence method (Li *et al*., 1994). A 16 mg sample of living ant was mixed with pre-cooled 0.8 mL of 0.05 M phosphate buffer (pH 7), and homogenized on ice-bath, followed by centrifugation at 14000 rpm for 45 min under 4ºC. The supernatant was the enzyme extract. A photo-chemical reaction took place in 3 mL reaction solution which contained 50 mmol/L) phosphate buffer (pH 7), 13 mmol/L methionine, 75 mmol/L nitroblue tentrazolium (NBT), 0.1 mmol/L ethylene diamine tetraacetic acid (EDTA) and 0.5 mL enzyme extract. The solution was added 4 umol/L lactoflavin and reacted for 15 min under the fluorescence room light at 25ºC. The photo-chemical reaction was stopped with darkness and the optical density of the solution was measured using 721 colorimeter at 560 nm. One enzyme unit is equivalent to the amount of the enzyme that caused half of 3 mL reaction solution to be inhibited.

Mineral analysis

According to the method of Mao *et al*. (2002), 2.0 g of ant powder was digested and then dissolved in 30 mL of HNO_3, 2 mL of H_2O_2 and 5 mL of $HClO_4$. The residue was diluted with 10 mL of double-distilled (dd) H_2O and filtered; the solution was diluted with dd H_2O to a volume of 150 mL. The concentration of the elements in the ant was determined with Plasma-spec ICP-AES (Leeman, American), using the calibration curves of standard elements. Selenium was determined by Fluorescence Spectrometry according to method by Li and Yu (1997).

Supercritical CO_2 fluid extraction of the ant soluble organic compounds

Supercritical CO_2 fluid system (SFE-CO_2, HL-1/32-50Mpa) was used to extract the organic soluble compounds. The temperatures and pressures of the extraction and liberation vessels were 50ºC and 30 Mpa, and 45ºC and 8 Mpa, respectively. The linear velocity of CO_2 was maintained at 8 kg/h.

GC-MS analysis of the ant organic compounds

The ant organic soluble compounds were identified by Gas Chromatography (Agilent 6890 N)/Mass Spectrometry (Spectrometry 5973) with Wiley/NBS 98 Chemical database. A 1 μL sample of the extract from SFE, which had been diluted 10 times with dichloromethane, was injected into a fused silica column (30.0 mm × 0.25 mm × 0.25 μm), at split mode (1:1), with injector temperature of 280ºC. Helium was used as the carried gas at a constant linear velocity of 1 mL/min. Oven temperature was programmed from 80 to 195ºC at a ramp rate of 4ºC/min. The initial and final hold times were 1 and 20 min, respectively. The MS interface temperature was set at 280ºC. Electron impact (EI) ionization source temperature was 230ºC, the ionization voltage was 70eV, and the MS mass range was between 10-55 units. Identifications were made by matching the mass spectra of the unknowns in the sample with those in the database. The relative contents of soluble organic compounds were quantified based on their peak areas.

Results and Discussion

Concentration of nutritional components

The concentration of nutritional components of the ant is shown in Table 1. Protein and fat contents of the dried ant were >40% and <10%, respectively. These nutritional values should meet the need of the people who require high protein and lower fat in their diet. The ant contained 77000 IU/100g SOD and 2.6±1.8 mg/kg vitamin

Table 1. The contents of nutritional components of *P. vicina* Roger.

Component		Content*
Moisture	(%)	6.0±1.2
Protein	(%)	56.6±10.8
Fat	(%)	9.0±0.5
Vitamin E	(mg/kg)	2.6±1.8
Ash	(%)	6.2±3.1
Total acid	(%)	1.6±0.0

***Mean±SD; n = 3**

E. It was the first time SOD was detected in the edible ant. Anion radical (O_2^-) is one of the important age-associated factors. As one of the major antioxidant enzymes, SOD plays an important role in catalyzing the conversion of O_2^- to hydrogen and molecular oxygen, thereby helping to prevent tissue damage by O_2^- and its metabolites, and preventing the danger of Harber-Weiss reaction, which generates OH. In fact, SOD has been considered as one of the important anti-selenium factors (Holland *et al*., 2000). Therefore, SOD could be regarded as an important factor associated with the anti-aging function of the ant (Tian and Zhang, 2002). Previously, it had been reported that the ant contained more than 40% protein, and vitamin E, B_1, and B_2 (Li *et al*., 1994). The protein is a vital component of cells. Vitamin E serves as one of the body's main defenders against oxidative stress, protecting the polyunsaturated fats and other vulnerable components of the cells and their membrane from destruction (Sizer and Whitney, 2000). Protein and vitamin E concentrations are used as important quality indices of ant health products in China. Moreover, the ant contained total acid (calculated as formic acid), which was considered one of the specific functional components of Chinese ant health products. Wu (1994) reported that formic acid has bioactivity for rheumatoid arthritis.

Amino acid analyses

As shown in Table 2, the ant contained 52.58% (w/w) total amino acid. Among the amino acids, 18 were determined, and the most predominant ones were glutamic acid, glycine and aspartic acid. This was in agreement with those reported by Li *et al*. (1995) and Cai *et al*. (1993). The ratio of essential amino acids to total amino acids is 0.35, and of essential amino acids to non-essential amino acids is 0.54. The determination of amino acid profile of the edible black ant is of great value from a nutritional standpoint.

Table 2. Amino acid content of *P. vicina* Roger.

Amino Acids		Content (%)*
Essential amino acids		
Threonine	Thr	2.26±0.69
Valine	Val	3.43±1.03
Methionine	Met	1.19±0.37
Isoluecine	Ile	2.26±0.69
Leucine	Leu	3.92±0.41
Lysine	Lys	2.20±0.47
Phenylalanine	Phe	1.76±0.06
Tryptophan	Trp	1.12±0.72
Cysteine	Cys	ND
Total essential amino acids (EAA)		18.50±1.81
Non essential amino acids		
Aspartic acid	Asp	5.05±1.02
Serine	Ser	2.94±0.50
Glutamic acid	Glu	7.45±1.61
Glycine	Gly	5.69±1.30
Alanine	Ala	4.54±0.75
Tyrosine	Tyr	2.82±0.35
Arginine	Arg	2.73±0.36
Proline	Pro	2.83±0.50
Histidine	His	3.39±1.67
Total non essential amino acids (Non EAA)		34.08±4.14
Total amino acids		52.58

*** Mean±SD; n = 3**

Note: Data on cysteine are not included in this work since this amino acid is destroyed during acid hydrolysis.

Table 3 shows the essential amino acid pattern in the ant as compare with FAO/WHO 1973 pattern. Food protein quality rating is based on the ten essential acids shown in Table 3. Results in Table 3 showed that the overall amino acid score (AAS) of the ant (x100) was 93.15. Methionine + cysteine had the lowest AAS (0.54), thus seemed to be the first limiting essential amino acids. Lysine

Table 3. Evaluation of the essential amino acid score of *P. vicina* Roger.

Amino Acids	ILe	Leu	Lys	Met+Cys	Phe+Tyr	Thr	Trp	Val	Total
Content (%)	2.26	3.92	2.20	1.19	4.58	2.26	1.12	3.43	20.96
Amino acid score	0.90	0.89	0.65*	0.54**	1.21	1.05	1.87	1.11	93.15
FAO/WHO 1973 pattern (%)	2.50	4.40	3.40	2.20	3.80	2.50	0.60	3.10	22.50

**** First limiting amino acid**
*** Second limiting amino acid**

Note: Amino acid scores (AAS) = Content of essential amino acid of test protein (%)/Content of same amino acid of FAO/WHO pattern (%)
Total AAS is calculated from: [Total essential amino acid content of test protein (%) / Total essential amino acid content from FAO/WHO 1973 pattern (%)] x 100
Methionine + cysteine has the lowest amino acid score and thus they are considered to be the first limiting essential amino acids.

Table 4. Mineral content of *P. vicina* Roger.

Mineral	Content (mg/kg)*
Calcium	1754.0±543.1
Potassium	4481.8±846.4
Magnesium	1030.5±20.5
Phosphorus	1579.5±623.0
Zinc	227.0±86.7
Manganese	210.0±70.7
Iron	940.5±285.0
Selenium	0.5±0.4
Copper	23.7±12.5
Barium	29.4±9.9
Chromium	17.1±0.9
Silicon	14.8±6.2
Nickel	7.2±2.3
Sodium	1433.3±245.4
Lead	1.3±0.2
Arsenic	0.6±0.1

*** Mean±SD; n = 3**

with the AAS of 0.65 was the second limiting amino acid. The quality of a food protein depends largely on its amino acid content. The cells, in making their own protein, need a full array of amino acids from food. Cells can synthesize non-essential amino acids when they are unavailable from food, but essential amino acids can only be obtained from foods (Sizer and Whitney, 2000). The high scores of essential amino acids present in the ant implied that it has a high biological protein value.

Mineral content

As shown in Table 4, the ant contained 16 minerals, of which K, Ca, P, Mg, Fe, Mn and Zn were the most predominant. Previous reports (Li *et al*., 1994; Cai *et al*., 1997; He and Li, 2003) identified more than 20 minerals in the ant. Minerals are one of the most important components of the ant because of their functional properties. For example, Fe is related to hematopoiesis; Mn is related to hematopoiesis, metabolism and skeletal

growth; Mg and Cu are essential elements in metallic enzyme; Zn could promote development and enhance immunity (Sizer and Whitney, 2000). Zn and Mn are considered the most important elements because they are related to the immunity regulation and anti-aging functions of the ant (Wu, 1994).

However, it should be noted that the ant powder contained substantial amount of lead (1.3 mg/kg) and arsenic (0.6 mg/kg). According to the Health Food Standard of GB 1670-1997, the lead contents of capsulated products and other health food products should be lower than 1.5 and 1.0 mg/kg, respectively, and the arsenic 1.25 and 0.3 mg/kg, respectively (Tian and Zhang, 2002). In order to guarantee safety, all ant products marketed as health food in China are required to provide safety test reports on their chemical analysis, hygiene and safety evaluation. A safety test on the acute and subchronic toxicity (Li *et al*., 1995) showed that the ant powder LD_{50} was >10g/kg. Its mutagenesis demonstrated by *in vivo* and *in vitro* tests was negative. According to the standard of GB 15193.3-1994, the ant powder sample was considered nontoxic.

Soluble organic compounds and their composition

The orthogonal experiments using SFE-CO_2 yielded 13.2% extract from the dried ant powder (sampled from Hangzhou suburb). Figure 1 and Table 5 showed that 24 soluble organic compounds from ant SFE extract were identified and quantified. The main compounds were 9-octadecenoic acid [E], ethyl oleate, cholesterol, n-hexadecanoic acid, 13,17-dimethylhentriacontane, and heptadecane, 9-octyl, with relative concentration of 45.10%, 9.30%, 6.00%, 4.96%, 2.09% and 2.04%, respectively. More than 20 volatile components have been determined from the organic extract as reported by Li *et al*. (1995) and Wang *et al*. (1997). In the present study, 6 compounds (hexadecanoic acid, linoleic acid, ethyl esert, ethyl oleate, oleic acid, cholesterol and cholesta-3, 5-diene) have not been reported previously. Ant extract contained 64.58% unsaturated fatty acids comprised of 8 components including hexadecenoic acid, n-hexadecanoic acid, ethyl, 9-hexadecenoate, hexadecanoic acid, ethyl ester, 9-octadecenoic acid, linoleic acid, ethyl oleate and oleic acid. Fats have many physiological functions, e.g. serve as the body's main form of stored energy, form the major material of cell membranes, and are converted to other physiological compounds. However, as reviewed by Li (2003), over-consumption of saturated fatty acid was harmful to health because it could cause diseases, such as cardiovascular disease and diabetes. Increase intake of polyunsaturated fatty

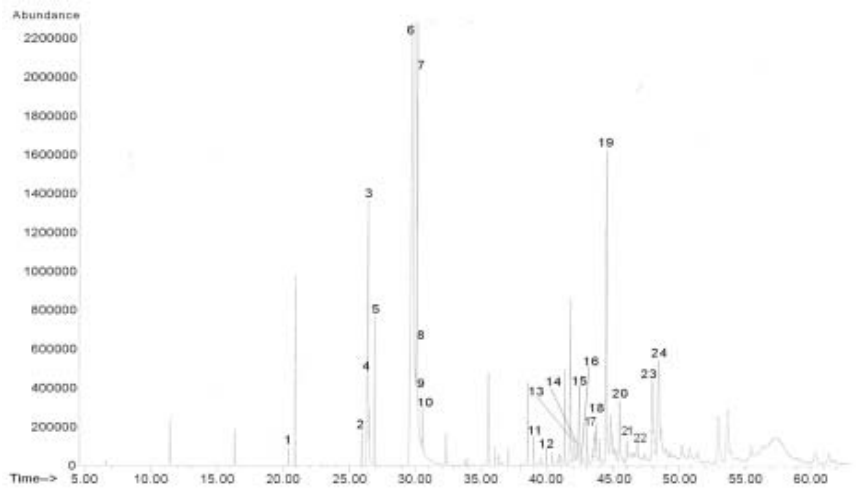

Figure 1. Chromatogram of the soluble organic compounds of the ant extract using SFE-CO_2

Table 5. Soluble organic components of the ant extract using SFE-CO_2.

Peak No	Components	Molecular Formula	Relative Contents (%)
1	8-Heptadecane	$C_{17}H_{34}$	0.12
2	Hexadecenoic acid, [Z]	$C_{16}H_{30}O_2$	0.44
3	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	4.96
4	Ethyl-9-hexadecenoate	$C_{18}H_{34}O_2$	0.78
5	Hexadecanoic acid , Ethyl ester	$C_{18}H_{36}O_2$	1.44
6	9-Octadecenoic acid[E]	$C_{18}H_{34}O_2$	45.10
7	Linoleic acid : 9,12-Octadecadienoic acid [Z,Z]	$C_{18}H_{32}O_2$	1.15
8	Ethyl oleate	$C_{20}H_{38}O_2$	9.30
9	Oleic acid	$C_{18}H_{34}O_2$	1.41
10	Octadecadienoic acid, Ethyl ester	$C_{20}H_{40}O_2$	0.43
11	Pentadecane,8-hexyl	$C_{21}H_{44}$	0.48
12	Heptadecane,3-methyl	$C_{18}H_{38}$	0.17
13	1-Hexacosen	$C_{26}H_{52}$	0.53
14	1-Octadecanethiol	$C_{19}H_{39}SH$	0.24
15	10-Methylnonadecane	$C_{20}H_{42}$	0.33
16	9-Nonadecene	$C_{19}H_{38}$	0.28
17	Nonacosanol	$C_{30}H_{62}O$	0.37
18	1-Docosene	$C_{22}H_{44}$	0.51
19	Cholesterol : 7-(1,5-Dimethylhexyl)-10,13-dimethyl-2, 3,4,7,8,9,10,11, 12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a] phenanthren-3-o l	$C_{27}H_{46}O$	6.00
20	Cholesta-3,5-diene	$C_{29}H_{46}$	1.04
21	Nonadecane, 9-methyl	$C_{20}H_{42}$	0.41
22	1-Octadecanethiol	$C_{18}H_{37}SH$	0.23
23	13,17-Dimethylhentriacontane	$C_{33}H_{68}$	2.09
24	Heptadecane,9-octyl	$C_{25}H_{52}$	2.04
	Straight chain alkyl hydrocarbon compounds		16.33

acid is beneficial as it can reduce blood lipid, blood pressure, and improve insulin resistance. Therefore, high polyunsaturated fatty acid content in the ant, may contribute to its health benefits.

Conclusion

Results from the present study indicate that *P. vicina* Roger is rich in nutrients including protein, fat, vitamin E and SOD, especially essential amino acids, unsaturated fatty acids and minerals. Therefore, the ant could be considered a potential health food and may be of use to the food industry as a source of ingredients with high nutritional value.

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