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Full Paper

Nutritional and fatty acid profiles of sun-dried edible black ants (*Polyrhachis vicina* Roger)

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Abstract: Determination of the nutritional composition of sun-dried edible black ants (*Polyrhachis vicina* Roger) cultivated in Zhejiang and Guizhou Provinces, China, was carried out. The Zhejiang and Guizhou ants contained 31.5% and 41.5% protein, 15.7% and 15.9% lipid, and 25.4% and 26.4% fibre respectively. Monounsaturated fatty acids were the most predominant fatty acids (71.4–72.7% of total fatty acids) found in both ant samples, followed by saturated fatty acids (23.8–25.5%) and polyunsaturated fatty acids (3.1–3.7%). A significant amount of n-3 fatty acids was detected: 87.4 mg/100g and 145.6 mg/100g in Zhejiang and Guizhou ants respectively. Phosphorus, iron and calcium were the main minerals found in the ant samples. A small amount of selenium was also found.

Keywords: edible black ants, *Polyrhachis vicina*, nutritional composition

Introduction

Edible black ants (*Polyrhachis vicina* Roger) are widely distributed in China. However, they are cultivated mainly in Zhejiang and Guizhou Provinces in southern China. They have been made into various products such as wines, powders and capsules. Some of these products have been exported to South Korea, Japan, Thailand and other South-East Asian countries [1].

Globally, there are more than 500 edible insect species, the most popular being ants, grasshoppers, silk worm pupae, locusts, beetles, crickets and bamboo worms [2-3]. Edible black ants

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have been used as human food and health food for hundreds of years in China [4]. They are an important cheap source of high-protein food in rural areas. In China, edible black ants have been extensively studied and shown to have many pharmacological properties such as anti-cancer, anti-fatigue and anti-inflammatory [5].

Sun drying is a common traditional method for drying fresh ants; it is still used in edible black ant processing industries in China. The fresh ants are put in water or in a freezer at -20°C and then were sun dried. While a few studies have investigated the nutrition composition of hot-air-dried black ants [6–7], for sun-dried ants which are commonly eaten, there is limited information on their nutritive value. In the present study, therefore, the determination of the nutritional composition of sun-dried edible black ants is carried out.

Materials and Methods

Sample preparation

The cultivated adult edible black ants (*Polyrhachis vicina*) were collected in Zhejiang and Guizhou Provinces, China, in June 2006. The fresh ants were stored at -20°C overnight, then sun dried for 3 days (10 h/day) and powdered with mortar and pestle until homogeneous.

Sampling

For each experiment, three samples were randomly collected from different batches of sun drying.

Proximate analysis

All solvents used were of analytical grade or HPLC grade and were purchased from Merck (Darmstadt, Germany) or Sigma-Aldrich (Steinheim, Germany). Crude protein, crude lipid, ash, crude fibre and moisture content were determined using the standard methods of the AOAC [8]. Crude protein was determined by the micro-Kjeldahl procedure; the factor N × 6.25 was used to convert nitrogen into crude protein. Results were calculated as per cent of dry weight (DW). Crude lipid content was obtained by the Soxhlet extraction method using petroleum ether as solvent. Ash content was obtained by heating the sample at 550°C to constant weight. Moisture content was determined by heating the sample to constant weight at 105°C. Crude fibre was determined gravimetrically after chemical digestion and solubilisation of other materials present. The fibre residual weight was then corrected for ash content after ignition. Carbohydrate content was estimated by subtracting the sum of the weights of crude protein, crude lipid, ash and crude fibre from the total dry matter.

Amino acid analysis

Determination of amino acid profile was conducted according to the standard AOAC methods [8]. The ground ant sample was hydrolysed in 6 N HCl at 110°C under nitrogen atmosphere for 24 h. The hydrolysed solution was used in the determination of free amino acids with a Hitachi L8800

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(Hitachi, Japan) automatic amino acid analyser. The results were reported as milligrams of amino acid per 100 g of protein.

Fatty acid analysis

An oil sample for fatty acid analysis was obtained according to a previous study with modification [9]. Approximately 2 g of well-ground sun-dried ant sample were extracted with 20.0 mL of chloroform–methanol (2:1,v/v) containing 10 mg/L of butylated hydroxyl toluene (BHT) and 0.9 mg/mL of C19:0 (nonadecanoic acid) as internal standard. Then, the mixture was stored in the fume hood for 24 h, then filtered and transferred to a separating funnel and added with 0.9% sodium chloride (10 mL). After shaking, the phases were allowed to separate and the separated lower phase was then concentrated at 38°C and transferred to a 10-mL volumetric flask and made up to volume with chloroform containing 10 mg/L of BHT. The total oil content was determined by evaporating off the solvent at 38°C under N_2 to constant weight.

The fatty acid methyl esters (FAMEs) of the total lipid extract were prepared by transesterification using 0.9M H_2SO_4 in methanol [10]. Briefly, lipid solution (1 mL), 0.9M H_2SO_4 in methanol (3 mL) and toluene (1 mL) were added to a Teflon-capped tube and the mixture shaken strongly, then submerged in water bath at 70°C for 2 h. Then n-hexane (2 mL) and 0.9% sodium chloride (1 mL) were added to the tube and centrifuged at 1200 rpm for 15 min. The supernatant was then dropped into water (2 mL) and, after separating off the aqueous phase, dried with a little sodium sulphate anhydrous. The crude FAMEs solution obtained was filtered through a Sep-pak silica column (Alltech Associates, Inc., Deerfield, IL) before injection into the gas chromatograph, which is a Shimadzu GC-14C system equipped with a flame ionisation detector (Shimadzu Corp., Kyoto, Japan), a fused silica capillary column (DB-23, 60m×0.248mm×0.25μm: Agilent Technologies, Inc., Palo Alto, CA, USA) and the N2010 Chromatography Data System (Zhida Information Technologies, Inc., Hangzhou, China). Injection and detection temperature was 270°C and 270°C respectively. The column temperature was kept at 100°C for 3 min and programmed to 190°C at a rate of 20°C/min and kept at 190°C for 10 min. It was then increased to 205°C at a rate of 5 °C/min and kept at 205°C for 6 min. Finally, it was increased to 230°C at a rate of 10°C/min and kept at 230°C for 5 min. Individual fatty acids were identified by means of purified standards (Sigma-Aldrich, Deisenhofen, Germany) and quantified by means of the internal standard method [11].

Mineral analysis

Sun-dried ant samples were subjected to acid digestion and analysed according to the procedure described by Farías et al. [12] with modification. A sample of ant powder (2 g) was ashed in a muffle furnace for 4 h at 550°C. After cooling, HNO_3 (30 mL), H_2O_2 (2 mL) and $HClO_4$ (5 mL) were added. Then the mixture was boiled to near dryness. The residue was added with doubly distilled water (10 mL) and filtered. Then it was diluted with doubly distilled water to a volume of 100 mL and used for analysis. Mineralogical analysis was carried out using ICP emission spectroscopy (IRIS Intrepid II XSP,

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Thermo Elemental Corporation, USA). All determinations were performed in triplicate and data represented on a dry weight basis as mean values ± standard deviation.

Statistical analysis

All experiments were performed in triplicate. The design was completely randomised [13]. The data were compared by one-way analysis of variance (ANOVA), and the mean values were compared by least squares difference method with SPSS 16.0 program. Significance was set as $p<0.05$.

Results and Discussion

Proximate composition

The proximate composition of the sun-dried edible black ants is shown in Table 1. Similar to previous report [7], both Zhejiang and Guizhou ants are low in moisture, moderate in lipid and fibre, and high in protein. However, the ants from the two locations are significantly different ($p<0.05$) with respect to the moisture, protein and carbohydrate content. Low moisture content means a good shelf life characteristic while the most abundant component is protein, indicating that these sun-dried ants are a good protein supplement. The protein content of the Guizhou ants is significantly higher than that of the Zhejiang ants ($p<0.05$), while the reverse is true for moisture content. It is possible that a slight difference in sun-drying conditions might have led to different dehydrating rates for the two ant samples, resulting in a difference in protein content [14].

High crude fibre in food is known to promote digestibility and enhance health benefits such as reduction of the risk of gastrointestinal cancers [15]. From Table 1, fibre is the second major component in both ant samples while lipid is the third most abundant component (about 15%). Hot-air-dried Hangzhou black ants contained 9.0% of lipid [7]. Thus, lipid loss may be more intensive in hot-air-dried ants than that in sun-dried ants. Lipid may be lost throngh moisture evaporation during hot-air drying and extensive heat treatment also appears to increase lipid loss phenomenon [16]. The quantity of ash in ant samples supposedly represents the amount of nutritionally important minerals [17]. From Table 1, the ash content is significantly higher in Guizhou ants than that in Zhejiang ants.

Table 1. Proximate composition of sun-dried edible black ants

Component (g/100g)	Zhejiang sun-dried ants	Guizhou sun-dried ants
Moisture	12.8 ± 0.1^{b}	8.6 ± 0.1^{a}
Crude protein	31.5 ± 0.5^{a}	41.5 ± 1.2^{b}
Crude fibre	25.4±0.9	26.4±1.4
Crude lipid	15.7±0.1	15.9±1.1
Crude carbohydrate	12.4 ± 1.1^{b}	3.8 ± 1.2^{a}
Ash	2.2 ± 0.1^{a}	3.8 ± 0.1^{b}

Note: Values are means ± standard deviations (n=3). Those having different superscript letters in a row differ significantly at $p<0.05$.

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Amino acid profile

The amino acid composition of the sun-dried ants is shown in Table 2. Eighteen amino acids were detected in both Zhejiang and Guizhou ants, similar to previous reports for hot-air-dried ants [6-7]. The major amino acids in both Zhejiang and Guizhou sun-dried ant proteins are glycine and glutamic acid, and the least abundant ones are cysteine and methionine. The proportion of a few amino acids (threonine, valine, lysine, phenylalanine, tryptophan, serine, glycine, arginine, proline and histidine) seems to be significantly influenced by the location of the ants. Ants need a nutritional source of arginine, histidine, leucine, isoleucine, lysine, methionine, phenylalanine, threonine, trythophan and valine, which is the same as for human young [18]. Total essential amino acid content in Guizhou ants is significantly higher than that in Zhejiang ants, which may be attributable to difference in amino acid content in the feed for the ants. Nevertheless, the amino acid profiles of the Zhejiang and Guizhou sun-dried ants are similar, their essential amino acid content being 34.6% and 36.1% respectively of the total protein, which is similar to that in egg (38.8% of total protein) [19] but substantially higher than that of Hangzhou hot-air-dried ants (18.1% of total protein) [7]. Also, the protein of the sun-dried ants has a higher proportion of methionine, valine, lysine, threonine, isoleucine, leucine, phenylalanine and tryptophan than that of the Hangzhou hot-air-dried ants. These results seem to imply that there is a protein and amino acid loss probably caused by the Maillard reactions during the hot-air drying process [20] whereas sun drying does not induce as much loss of amino acids in the ants.

Fatty acid composition

The sun-dried ants are rich in lipid. The composition and concentration of fatty acids in the sun-dried ant oil are presented in Tables 3-4. Twenty-two and 23 fatty acids are identified in Guizhou and Zhejiang ant oil respectively. Unsaturated fatty acids vary between 74.5-76.4% with monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) ranging between 71.4-72.7% and 3.1-3.7% respectively. As a result, sun-dried ant samples could be a good source of unsaturated fatty acids. The 16:0 fatty acid is found as the major saturated fatty acid while the 18:1 acid is the main MUFA (60.5-63.0%).

The n-3 PUFA are known to prevent cardiovascular disease [21] and are anti-inflammatory [22]. Total PUFA content (n-3 + n-6) is higher in Guizhou than in Zhejiang ants. Since both groups of ants feed on similar diet (such as insects, larvae and algae), the difference, though small, in the proportions of their PUFA and total unsaturated fatty acids could possibly be attributed to the climate factor [23-25], the average annual temperature in Guizhou Province being lower than that in Zhejiang Province. The 18:3(n-3) is the main n-3 PUFA in both Zhejiang and Guizhou ants, amounting to 63.0 mg and 123.5 mg per 100 g of total fatty acids respectively. With the quantities of total n-3 PUFA being as they are (Tables 3-4), the Zhejiang and Guizhou sun-dried ants can be utilised as a source of these fatty acids. It has been recommended that human beings evolve on a diet with n-3/n-6 ratio of approximately 1 [26-27]. High ratio of n-3/n-6 is advantageous for reducing the risk of cardiovascular diseases [26-27]. In this study, the ratio of n-3/n-6 is found to be 0.41 and 0.42 for Zhejiang and Guizhou sun-dried

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ants respectively. These ants are also a good source of unsaturated fatty acids which are beneficial to human health.

Table 2. Amino acid composition (mg/100g crude protein) of edible black ant samples

Amino acid	Zhejiang sun-dried ants	Guizhou sun-dried ants	Hangzhou hot-air-dried ants (Shen et al.[7])	Egg (Wang [19])
Essential amino acids				
Threonine	4016.7±1.3[a]	4263.3±1.3[b]	2260.0	4470.0
Valine	6883.3±1.5[a]	8273.3±1.4[b]	3430.0	5420.0
Methionine	1626.7±1.3	2326.7±1.1	1190.0	2810.0
Isoleucine	4650.0±2.1	4570.0±2.0	2260.0	4880.0
Leucine	6973.3±2.1	6980.0±2.0	3920.0	8110.0
Lysine	4370.0±1.7[b]	3736.7±1.4[a]	2200.0	6590.0
Phenylalanine	3340.0±1.9[a]	3496.7±1.1[b]	1760.0	4820.0
Tryptophan	2736.7±1.4[b]	2460.0±1.5[a]	1120.0	1720.0
Total essential amino acids	34596.7±1.6[a]	36106.7±2.2[b]	18140.0	38820.0
Non-essential amino acids				
Aspartic acid	7516.7±1.6	7756.7±1.8	5050.0	8920.0
Serine	4823.3±0.8[a]	5106.7±1.3[b]	2940.0	6720.0
Glutamic acid	11206.7±1.6	11570.0±1.4	7450.0	12130.0
Glycine	12860.0±1.3[b]	11596.7±2.1[a]	5690.0	3020.0
Alanine	9483.3±1.5	8843.3±1.0	4540.0	5030.0
Tyrosine	5340.0±1.7	5330.0±1.2	2820.0	3810.0
Arginine	3523.3±1.6[a]	3790.0±1.0[b]	2730.0	5700.0
Proline	7366.7±1.0[b]	6766.7±1.1[a]	2830.0	3380.0
Cysteine	696.7±1.5	730.0±1.5	3390.0	2090.0
Histidine	2580.0±1.2[b]	2396.7±2.0[a]	ND	1900.0
Total non-essential amino acids	65396.7±1.6[b]	63886.8±1.8[a]	37440.0	52700.0

Note: Values are means ± standard deviations (n=3). Values having different superscript letters in a row differ significantly at $p<0.05$.

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Table 3. Fatty acid composition (as % of total fatty acids) of sun-dried edible black ants

Fatty acid	Zhejiang ants	Guizhou ants
12:0	0.7±0.3	0.6±0.3
14:0	0.6±0.1	0.6±0.1
15:0	0.2±0.1	0.2±0.1
16:0	19.0±1.1	17.6±0.2
17:0	0.1±0.1	0.1±0.1
18:0	4.3±0.2	4.1±0.5
20:0	0.2±0.1	0.3±0.1
22:0	0.3±0.1	0.3±0.1
24:0	0.1±0.1	ND
Total SFA	25.5±1.0	23.8±1.8
14:1	0.1±0.1	0.1±0.1
15:1	0.2±0.1	0.1±0.1
16:1	8.9±0.2	8.2±0.2
17:1	0.5±0.4	0.3±0.1
18:1	60.5±0.8	63.0±0.3
20:1	0.1±0.1	0.1±0.1
22:1	1.1±0.2	0.9±0.1
Total MUFA	71.4±1.3	72.7±1.2
16:2 (n-6)	0.3±0.1	0.3±0.1
18:2 (n-6)	1.7±0.1	2.0±0.1
20:2 (n-6)	0.1±0.1	0.1±0.2
20:4 (n-6)	0.1±0.1	0.2±0.1
Total *n*-6	2.2±0.7	2.6±0.8
18:3 (n-3)	0.6±0.2	0.9±0.1
20:5 (n-3)	0.2±0.2	0.1±0.1
22:5 (n-3)	0.1±0.1	0.1±0.1
Total *n*-3	0.9±0.3	1.1±0.4
Total PUFA	3.1±0.6	3.7±0.7
PUFA:SFA	0.12	0.16
n-3/n-6	0.41	0.42

Note: Values are means ± standard deviations (n=3); ND = not detected, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids

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Table 4. Fatty acid concentration (mg/100 g) in sun-dried edible black ant oils

Fatty acid	Zhejiang sun-dried ant oil	Guizhou sun-dried ant oil
12:0	66.1±1.8	80.0±1.2
14:0	64.6±1.0	82.3±1.6
15:0	24.0±1.7	23.1±1.9
16:0	1935.7±1.4	2410.7±1.4
17:0	12.8±1.2	17.8±1.7
18:0	441.0±1.3	562.8±1.2
20:0	22.1±1.6	40.7±1.9
22:0	30.9±1.9	34.5±1.6
24:0	5.5±1.3	ND
Total SFA	2602.7±1.4	3251.9±1.2
14:1	11.8±1.8	8.2±1.1
15:1	16.5±1.1	7.3±0.6
16:1	898.9±1.6	1114.8±1.7
17:1	47.6±1.6	41.8±1.8
18:1	6118.9±1.6	8612.8±1.6
20:1	9.8±1.4	11.1±1.6
22:1	111.2±1.7	127.1±1.8
Total MUFA	7214.7±1.8	9923.1±1.3
16:2n-6	24.8±1.4	39.1±1.2
18:2n-6	169.0±1.3	276.8±1.4
20:2n-6	7.3±1.7	12.9±1.3
20:4n-6	14.3±1.4	21.2±1.5
Total *n*-6	215.4±1.9	350.0±1.2
18:3n-3	63.0±1.7	123.5±1.5
20:5n-3	17.6±1.5	13.9±1.7
22:5n-3	6.8±1.7	8.2±1.1
Total *n*-3	87.4±1.5	145.6±1.7
Total PUFA	302.8±1.2	495.6±1.7

Note: Values are means ± standard deviations (n=3); ND = not detected, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids

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Mineral composition

The mineral content of the sun-dried ants is shown in Table 5. The most abundant mineral in both sun-dried ants is phosphorus and the least abundant is selenium. Evidently, sun-dried ants are rich in phosphorus, iron and calcium. Iron content in Zhejiang ants is significantly higher than that in Guizhou ants while for calcium content the opposite is true. This may be ascribed partly to the difference in mineral content in their feeds. It is also noted that the relative proportion of the major minerals found in this investigation is substantially different from that obtained for oven-dried ants [7]. It can be concluded, however, that sun-dried ants are a good source of many important minerals such as phosphorus, calcium, iron and selenium. Iron can be antioxidants [28] while magnesium and zinc can prevent cardiomyopathy and growth retardation [29]. Selenium is an essential component in several major metabolic pathways including thyroid hormone metabolism, and also has other important biological functions such as antioxidative and immune regulating functions [30] and anti-cancer activities [31].

Table 5. Mineral content (mg/100g of sun-dried ants)

Mineral	Zhejiang ants	Guizhou ants	Hangzhou hot-air-dried ants [7]
Phosphorus	387.7±1.7	417.0±1.0	158.0
Iron	118.0±1.1^{b}	53.7±1.0^{a}	94.1
Calcium	49.1±0.7	108.0±0.4	175.4
Magnesium	65.3±1.3	67.6±1.0	103.1
Manganese	25.9±1.0^{a}	32.3±0.5^{b}	21.0
Zinc	17.6±1.1^{b}	11.9±0.1^{a}	22.7
Copper	2.4±0.5	1.9±0.1	2.4
Lead	0.4±0.1^{a}	0.6±0.1^{b}	0.1
Nickel	<2.0±0.1	<2.0±0.1	0.7
Chromium	<2.0±0.1	<2.0±0.1	1.7
Selenium	2.9x10^{-2}±0.1^{a}	3.8×10^{-2}±0.1^{b}	0.1

Note: Values are means ± standard deviations (n=3). Values having different superscript letters in a row differ significantly at $p<0.05$.

Conclusions

The present study has shown that Zhejiang and Guizhou sun-dried edible black ants are a rich source of protein, lipid, fibre and essential amino acids. In addition, they contain essential fatty acids such as 18:3 (n-3), 20:5 (n-3) and 22:5 (n-3) and are also rich in minerals such as phosphorus, iron, calcium, magnesium, zinc and selenium. Thus, they seem to have a potential in being used as a nutraceutical or alternative nutritional food source for humans.

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