

Studies on the Chemical Composition of the Ascomycete Fungus *Phaeangium lefebvrei* Pat.

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Abstract. Samples of ascocarps of the desert truffle *Phaeangium lefebvrei* Pat., commonly known as hober, were collected from Harrat Al-Harra, Northern Saudi Arabia and analysed for composition of total ash, moisture, protein, carbohydrates, fatty acids, mineral contents, crude fat, crude fibre, and free amino acids. They were found to contain 23% total protein (% dry weight), 18% total carbohydrates, 1% crude fat, and 3% total crude fibers. A total number of 26 amino acids (8 essential and 18 non essential) and 11 fatty acids were detected (8 saturated and 3 unsaturated). *Phaeangium lefebvrei* was also rich in minerals.

Introduction

Truffles (hypogeous ascomycetes) locally known as "Fagaa" or "Alkamah", are grown as a wild crop in almost half of the upper part of Saudi Arabia [1]. Five species have so far been reported from Saudi Arabia [1;2]. They are, *Tirmania nivea*, *T. pinoyi*, *Terfezia boudieri*, *T. claveryi* and *Phaeangium lefebvrei*. Their ecological distribution [1-3], and chemical analysis were also reported [4-6]. A summary of earlier investigation on Saudi Arabian truffles is given in Table 1 [1-12]. These truffles are rich in minerals, carbohydrates and proteins [4;5] and therefore are prone to attack by other fungi and bacteria which may cause spoilage [10]. Truffles are also reported to be edible, delicious and costly food around neighbouring countries of Saudi Arabia [13-15], in the continent Africa [16;17] and in the continent Europe [18-22].

Table 1. Previous studies on truffles in Saudi Arabia

	Reference	Truffles	Investigation	Ref. No.
1)	Abdallah <i>et al.</i> (1979)	Without Taxonomic identification	Minerals contents, Proteins, Sugar, Fats, Ascorbic acid and Riboflavin.	7
2)	Hussein & Eid. (1980)	Without Taxonomic identification	Mineral composition, Amino acids, Vitamins.	8
3)	Bokhary (1987) —	<i>Tirmania nivea</i> (Desf. ex Fr.) Trappe, <i>T. pinoyi</i> (Maire) Malencon, <i>Terfezia boudieri</i> Chatin	Occurrence, Identification and Distribution	1
4)	Bokhary <i>et al.</i> (1987)	<i>T. nivea</i> , <i>T. pinoyi</i> and <i>T. boudieri</i>	Carbohydrates, Proteins, Aminoacids, Mineral contents, Organic acids.	4
5)	Bokhary & Parvez (1987)	<i>T. nivea.</i> , <i>T. pinoyi.</i> , <i>T. boudieri</i> , <i>T. claveryi</i> and <i>Phaeangium lefebvrei</i> Pat.	Survey report on occurrence and Distribution	3
6)	Bokhary & Parvez (1988)	<i>Terfezia claveryi</i> Chatin, <i>Phaeangium lefebvrei</i> Pat.	Occurrence, Identification and Distribution	2
7)	Bokhary <i>et al.</i> (1989)	<i>T. nivea</i> , <i>T. pinoyi</i> , <i>T. boudieri</i>	Fatty acids	9
8)	Bokhary, Parvez (1989)	<i>T. nivea</i> , <i>T. pinoyi</i> , <i>T. boudieri</i> , <i>T. claveryi</i>	Spoilage Microflora	10
9)	Bokhary & Parvez (1992)	<i>T. nivea</i> , <i>T. pinoyi</i> , <i>T. boudieri</i> , <i>T. claveryi</i>	Soil mycoflora from under surface of these truffles ascocarps	11
10)	Bokhary & Parvez (1992)	<i>T. nivea</i> , <i>T. pinoyi</i> , <i>T. boudieri</i> , <i>T. claveryi</i> , <i>Phaeangium lefebvrei</i>	Soil mycoflora from undersurface of these truffles ascocarps	12
11)	Bokhary & Parvez (1993)	<i>T. claveryi</i>	Chemical composition	5
12)	Sawaya <i>et al.</i> (1985)	<i>T. nivea</i> , <i>T. claveryi</i>	Mineral composition, Moisture, Protein, Fat, Crude fibre, Ash, Ascorbic acid, Amino-acids.	6

Phaeangium lefebvrei was reported in the Arabian Peninsula by Alsheikh and Trappe [13] from Kuwait. In Saudi Arabia, *P. lefebvrei* was reported only from the northern part (Harrat Al-Harra) by Bokhary and Parvez [2;3]. *Phaeangium lefebvrei* is locally known as Hober and is commonly eaten by native and migrated birds but also by local inhabitants (beduins) [2;13]. *Phaeangium lefebvrei* was previously only analysed for total protein and amino acid contents [23]. Therefore, our objective was to analyse *P. lefebvrei* ascocarps for total ash, moisture, protein, carbohydrate, crude fat and crude fibre contents. In addition, mineral content, fatty acids and amino acids were determined.

Materials and Methods

Sample collection

Samples of *Phaeangium lefebvrei* ascocarps were collected from Harrat Al-Harra (Northern part of Saudi Arabia) in April 1987 [2]. This research was sponsored by National Commission for Wildlife Conservation and Development, Riyadh [3].

General chemical analysis

AOAC [24] standard methods for total moisture (method 7.003), total crude fat (method 7.056), and for total crude fibre (method 7.009) were followed.

Determination of minerals

For mineral analysis, samples were acid-digested with concentrated HNO_3 (65%) on a hot plate. Calcium, cobalt, copper, iron, magnesium, manganese, sodium and zinc were determined by flame absorption using a flame atomic absorption spectrophotometer (Pye Unicam SP9 equipped with SP9 computer, Phillips of Pye Unicam Ltd., Cambridge, U.K.). Potassium was determined by flame emission while phosphorus was determined colorimetrically [25] after digestion of tissue with concentration HNO_3 (65%) and 60% HClO_4 (20 ml HNO_3 + 5 ml HClO_4).

Determination of carbohydrates

Triplicate samples of ascocarps (5 g) were cut into small pieces, washed thoroughly to remove off attached soil and blotted dry. These samples were subsequently refluxed in three changes of boiling 80% ethanol. The bulb extract was dried under vacuum and dissolved in 30 ml hot water. The extract was then passed through cation (Dowex 50W-8X) 200-400 mesh and anion (Dowex 1W-8X) 200-400 mesh ion exchange resin columns [26;27], reduced to dryness and taken up in 10 ml 80% ethanol or water to study carbohydrate; amino acid and protein contents.

Qualitatively carbohydrate in the soluble and hydrolysed fraction after passing extract through ion exchange resins, was analysed by paper chromatography using three solvents: n-propanol: ethyl acetate: water (7:1:2), ethyl acetate: acetic acid: water (14:3:3), and methyl ketone: acetic acid: boric acid (9:1:1) [28;29] and detected by using silver nitrate sodium ethoxide [30]. Quantitative estimation of soluble sugars was done by gas liquid chromatography (Pye Unicam, 204 chromatograph with DP 101 computing device) [4;27;31-34], by comparing with a chromatograph of known carbohydrates (Standards).

Determination of amino acids and protein

Amino acids were eluted with lithium citrate buffer (0.2 N, pH 2.2) and analysed using an LKB 4400 analyser (LKB Biochrom Ltd. England) which was fitted with a 4.6 mm × 27 cm stainless steel column packed with ULTROPAC 8 (LKB Biochrom) 8 + 0.5 µm cation exchange resin. Amino acids were then identified with the standard chromatograph of known amino acids. Total protein (N × 6.25) in the residue after ethanol extraction were estimated by the Lowry *et al.* method [35], using a spectrophotometer (Pye Unicam, SP 7500 uv/vis) at 660 nm.

Determination of fatty acids

Fatty acid was determined according to Kates [36]. Samples (5 g for each of five replicates) were crushed and extracted with chloroform: ethanol (2:1, v:v) at 40-60°C and washed three times with 0.02% CaCl₂ solution. Fatty acids were then extracted with ethyl ether after acidifying the hydrolysate with concentrated HCl to pH₄, dried under vacuum and redissolved in a small volume of chloroform (2 ml), and dried again at 45°C under vacuum. Methanolic-HCl (10 ml) was added to the residue and refluxed for 90 min. An additional 10 ml of methanol: water (v:v) was added and the fatty acid methyl esters were extracted with dimethyl ether and redried under a vacuum. The residue was dissolved in 0.2 ml dimethyl ether and 1 ml of benzene, and 2.5 ml of BrF₃ was then added and heated to 90-100°C for 30 minutes. The residue was allowed to cool (25°C) and then dissolved in 1 ml of deionized water. Fatty acids in the sample were then identified by varian vista 6500 GLC with a data system equipped with flame ionisation detector 401 by comparing with a chromatograph of known fatty acids (standard).

Results and Discussion

General chemical analysis

General chemical analysis of *Phaeangium lefebvrei* is summarised in Table 2. Total protein content was (23%) higher than reported for *T. claveryi* although total

carbohydrate (18%) was lower than *T. claveryi* [5]. Total moisture, carbohydrate, crude fat, and crude fibre contents were slightly lower than reported for other truffle species in Saudi Arabia [4-6] and elsewhere [15]. Protein contents of truffles varied considerably from 3.3-79% in reports from Saudi Arabia and elsewhere [4-7;14;16;17;37]. In general *P. lefebvrei* could be a rich source of protein and carbohydrate for human beings although at present it is mainly eaten by native and migrated birds [2;38].

Mineral content

Mineral content of *P. lefebvrei* ascocarp is summarised in Table 3. Potassium was present in the largest amount followed by phosphorus, sodium, magnesium and calcium. Micronutrients were present in fair amount as recommended dietary allowances by Food and Nutrition Board [39]. Macronutrients were present in slightly higher amounts as compared to earlier reports on other truffle species from Saudi Arabia [4-6].

Table 2. General analysis of *Phaeangium lefebvrei* ascocarp

General analysis	% of dry weight
Total ash	4.2 ± 0.1
Total moisture	61.5 ± 1.4
Total protein	23.2 ± 1.5
Total carbohydrate	18.3 ± 0.1
Total crude fat	1.3 ± 0.1
Total crude fibre	2.6 ± 0.1

± Standard deviation

Table 3. Mineral composition of *Phaeangium lefebvrei* ascocarp

Mineral	mg/100g dry weight
Ca	65 ± 8
Co	86 ± 10
Cu	5 ± 0.1
Fe	110 ± 1
K	1650 ± 39
Mg	137 ± 15
Mn	27 ± 6
Na	168 ± 16
P	510 ± 18
Zn	12 ± 1

± Standard deviation

Carbohydrate analysis

Carbohydrate analysis of *P. lefebvrei* ascocarp is summarised in Table 4. Glycerol was present at the highest level followed by glucose, mannitol, inositol, trehalose and fructose. Individual carbohydrates reported here are in general lower in quantity than reported for other truffle species from Saudi Arabia [4-6].

Fatty acid contents

Eight saturated (myristic, pentadecanoic, margaric, stearic, nonadecanoic, arachidic, heneicosanoic, and behenic) and three unsaturated fatty acids (Palmitoleic, oleic, and euric) were found in *P. lefebvrei* ascocarp (Table 5). Fatty acid content of *P. lefebvrei* were higher than in *Tirmania* and *Terfezia* truffle species reported from Saudi Arabia [4;5].

Table 4. Carbohydrate analysis of *Phaeangium lefebvrei* ascocarp

Carbohydrate	ug/g dry weight
Glycerol	70 ± 7
Glucose	32 ± 1
Fructose	4 ± 0.1
Inositol	8 ± 0.1
Mannitol	17 ± 1
Trehalose	5 ± 0.1

± Standard deviation

Table 5. Fatty acid contents of *Phaeangium lefebvrei* ascocarp

Fatty acid	% dry weight of lipid fraction
C14	9 ± 2
C15	3 ± 0.2
C16	-
C17	32 ± 3
C18	12 ± 1.5
C19	18 ± 2.5
C20	12 ± 1
C21	18 ± 0.8
C22	2 ± 0.2
C16:1	18 ± 1.3
C18:1	3 ± 0.8
C18:2	-
C22:1	17 ± 1.4

± Standard deviation

- Absent

Amino acid contents

Phaeangium lefebvrei contained a total of 26 amino acids, eight essential and eighteen non-essential (Table 6). This compared to 29 amino acids reported earlier from *Terfezia claveryi* [5], 23 amino acids from *Terfezia boudieri*, 22 amino acids from *Tirmania nivea* and 20 amino acids from *Tirmania pinoyi* [4]. Sawaya *et al.* [6] reported only 18 amino acids from *T. claveryi*. In *P. lefebvrei* glutamic acid was present in highest amount followed by arginine, tyrosine and leucine. Phosphoserine was present in the lowest amount. Amino acids like β -aminobutyric acid, carnosine, lysine, 1-methyl histidine, 3-methyl histidine, sarcosine, taurine and tryptophan which were reported from other truffle species [4;5] were absent. The quantity of essential amino acids were generally higher in *P. lefebvrei*, as compared to other truffle species [4-6].

Table 6. Amino acid contents of *Phaeangium lefebvrei* ascocarp

Amino acid	mg/100 g dry weight
Alamine	64 \pm 2
β -alanine	213 \pm 10
α -amino adipic acid	64 \pm 4
α -amino isobutyric acid	416 \pm 9
Anserine	393 \pm 15
Arginine	1175 \pm 33
Aspartic acid	895 \pm 15
Citruline	337 \pm 16
Cystathionine	497 \pm 19
Cystine	232 \pm 17
Ethanolamine	70 \pm 13
Glutamic acid	1546 \pm 19
Glycine	311 \pm 10
Histidine*	849 \pm 19
Leucine	1052 \pm 22
Isoleucine*	463 \pm 12
Methionine*	220 \pm 8
Ornithine	176 \pm 12
Phenylalanine*	708 \pm 12
Phosphoethanolamine	237 \pm 8
Phosphoserine	35 \pm 7
Proline	754 \pm 10
Serine	293 \pm 12
Threonine*	455 \pm 24
Tyrosine	1072 \pm 27
Valine	657 \pm 17

\pm Standard deviation

* Essential amino acid

Conclusion

The results of present study suggested that, although *P. lefebvrei* ascocarps are usually eaten by native and migrated birds, they could be a good source of nutrition for human beings too. This is the first report of chemical analysis of *P. lefebvrei* from Saudi Arabia.

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دراسات على التركيب الكيميائي للفطر الزقي فينجيم ليفبري بات

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(سُلِّمَ في ١١/٢٧/١٤١٤ هـ؛ وقُبِلَ للنشر في ١٠/٦/١٤١٥ هـ).

ملخص البحث . تمّ جمع عيّنات من الأجسام الثمرية للكمأة الصحراوية فينجيم ليفبري بات والمعروفة بالهوبر من حرّة الحرّة شمال المملكة العربية السعودية . كما تمّ تحليل تركيب كل من الرماد الكلي، الرطوبة، البروتين، المواد السكرية (الكربوهيدراتية)، الأحماض الدهنية، المحتوى المعدني، الدهن الخام، الألياف الخامة والأحماض الأمينية الحرّة . وقد وجد أنها تحتوي على ٢٥٪ بروتين كلي (النسبة المثوية للوزن الجاف)، ١٨٪ مواد كربوهيدراتية، ١٪ دهن خام و ٣٪ ألياف خامّة، كما تبين من البحث أن هذه الأجسام الثمرية تحتوي على ستة وعشرين حامضاً أمينياً (ثمانية أساسية، ثمانية عشر غير أساسية) بالإضافة إلى أحد عشر حامضاً دهنيّاً (ثمانية مشبعة، وثلاثة غير مشبعة) كما وجد أن هذا الفطر غني بالمعادن .