Arab Gulf J. Scient. Res., Agric. Biol. Sci., **B6** (1), pp. 65-73 (1988)

Biochemical Changes During Developmental Stages of *Phaeangium lefebvrei* Pat. Sporocarp

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ABSTRACT. *Phaeangium lefebvrei* Pat., is one of several truffles found in Iraq and other Arabian countries. It is called "Hobbar" locally. A brief description and camera lucida illustrations of this species are provided in this paper.

Polyacrylamide gel electrophoresis was used to determine the banding patterns of total soluble proteins at two developmental stages of sporocarps of this fungus. The patterns for total proteins of the sporocarp stages differed quantitatively, although there was close similarity with respect to the number of bands and position of the peaks. The immature stage showed a high protein content, indicating the high metabolic demands for formation of asci and ascospores.

The analyses showed that the tubers could be considered nutritionally as a good source of protein.

Phaeangium lefebvrei Pat. (locally known as "Hobbar") is one of several species of truffles found in Iraq, Kuwait, Saudia Arabia, Syria, other Middle East countries and North Africa. During our survey in the desert of south Iraq, we found that this species of truffles is associated with annual *Helianthemum* spp. and it probably forms a mycorrhizal association. The growth of this fungus is related to rainfall in early autumn (Oct. - Nov.).

As far as the authors aware, no previous study of the chemical composition and nutritional value of this species has been done. Al-Sheikh and Trappe (1983) devoted special attention to taxonomical and ecological studies on *Phaeangium lefebvrei* grown in Kuwait, and described it as a desert truffle eaten by birds. However, chemical analysis of other truffle species has been carried out by El-Gendy and Alami (1976), Al-Delaimy (1977), Hussen and Eid (1980) and Ahmed *et al.* (1981). Their studies revealed the presence of several minerals and

seventeen amino acids, including nine essential amino acids in samples with high protein contents.

The aim of this study was to determine the chemical composition and protein behaviour during development of this type of truffle in Iraq. Special attention has been focused on protein determination in relation to developmental stages using polyacrylamide gel electrophoresis.

Materials and Methods

Organism

The samples used as a source of the sporocarp stages were collected from the Iraqi desert (Safwan-Basrah desert) during January to March 1985. Twenty samples of each stage were examined to record morphological features of the fungus throughout the whole season.

Chemical Analysis

Fresh sporocarps of the required age "immature and mature" were collected from the soil, cleaned with the aid of knife to remove the adhering soil, weighed and used for moisture determination by heating in an oven at 60°C for 24 hr, cooled in a desiccator, and reweighed. The dried material was grounded in a mortar and used for amino acid analysis. The method of Mondino and Bongiovanni (1970) was adopted for measuring amino acids; 2 g. sample each were placed in a flask with 250 ml boiling 6N HCl. The mixture was hydrolysed under nitrogen atmosphere in constant boiling HCl at 110°C for approximately 24 hr. The mixture was then cooled to room temperature filtered, evaporated and washed the final filtrate was analysed on an LKB Biochrom 4101 amino acid analyzer.

Polyacrylamide gel electrophoresis was employed to study the banding patterns of total soluble proteins at different stages of development of the fungus. A mortar and pestle was used to grind the tissue before protein was extracted. Protein was determined after precipetation with 10% (W/V) trichloroacetic acid by the method of Lowry *et al.* (1951), using bovine serum albumin as a standard. The method of electrophoresis was derived from that of Ornstein and Davis (1964) with certain modifications by using "Shandon" analytical polyacrylamide electrophoresis apparatus. The instrument used for densitometry was the Unicam SP 1800 scanning densitometer fitted to an SP 1800B spectrophotometer, which used an AR 25 linear recorder. Peak area measurements were obtained using an LKB 2220 Integrator.

Results and Discussion

The chief diagnostic features of this species were 2-4 spored asci, long - stipitate when immature, and 8-spored asci, very short - stipitate at maturity (Table 1 & Fig. 1: A-D). It was concluded that these features generally fit the description given by Al-Sheikh and Trappe (1983) for *Phaeangium lefebvrei* Pat.

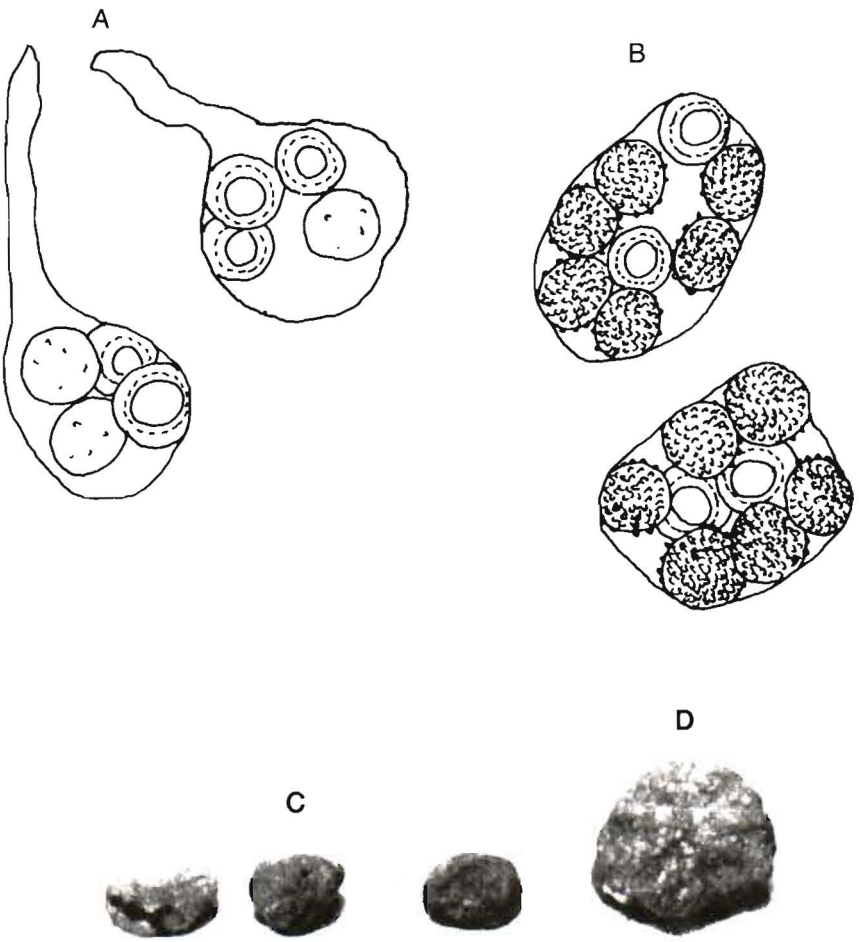

Fig. 1. A-D. *Phaeangium lefebvrei*

a. 4-Spored asci of immature sporocarp
b. 8-Spored asci of mature sporocarp
c. Fresh sporocarps of immature stage
d. Fresh sporocarp of mature stage

Table 1. Developmental stages of *Phaeangium lefebvrei* Pat. sporocarp

Maturity of sporocarps	Description
Immature	***Sporocarp:*** found in groups of four or five 4-5 mm × 3-5 mm, subglobose to irregular, light brown, smooth wall, lacking a basal tuft of hyphae. ***Gleba:*** solid, white in color. ***Asci:*** 2-4 spored, subglobose to obvoid, 55-62 × 75-82 µm, long stipitate, 3-6 × 40-92 µm. ***Spores:*** broadly ellipsoid to globose, 22-25 × 25-30 µm, hyaline and smooth.
Mature	***Sporocarp:*** 18-20 mm × 22-28 mm subglobose to irregular, dark brown, verrucose, covered with densely brown tomentose, lacking a basal tuft of hyphae. ***Gleba:*** solid, white with dark brown fertile pockets separated by white sterile veins. ***Asci:*** 8 - spored, ellipsoid, subglobose or irregular, not stipitate. ***Spores:*** ellipsoid to globose, 22-25 × 75-82 µm, the walls 1-2 µm thick and ornamented with crowded papillae, light olive in Melzer's reagent.

There is increasing need for knowledge about proteins since they are known to play an important role in morphogenesis of organisms. To study the proteins in *P. lefebvrei,* two development stages (Table 1) of sporocarp were analysed for soluble protein in 7.5% polyacrylamide gel. Figure 2 (A-B) shows similarities in their electrophoretic patterns with regard to the position of the peaks. The number of bands in each pattern was the same (8 band in each). However there were some quantitative differences between the two patterns (Table 3).

The immature stage demonstrated moderate to high intensity for most of the bands. This remarkable high protein content made at this early stage of development is worthy of note, and this may reflect the high metabolic demands made for the initiation of asci and ascospores within the fruiting body. Assuming that the pattern of protein synthesis reflects, at least partially, the outcome of a developmental programme, one could conclude that initiation of asci and ascospores formation induces a large number of biochemical processes. The function of these protein is unknown. Clare *et al.* (1968) suggested that the soluble fungal proteins have an active cellular function.

As the fruiting body matured, clear changes in the protein pattern were observed. Most of the peaks became lower except two bands (first and the sixth

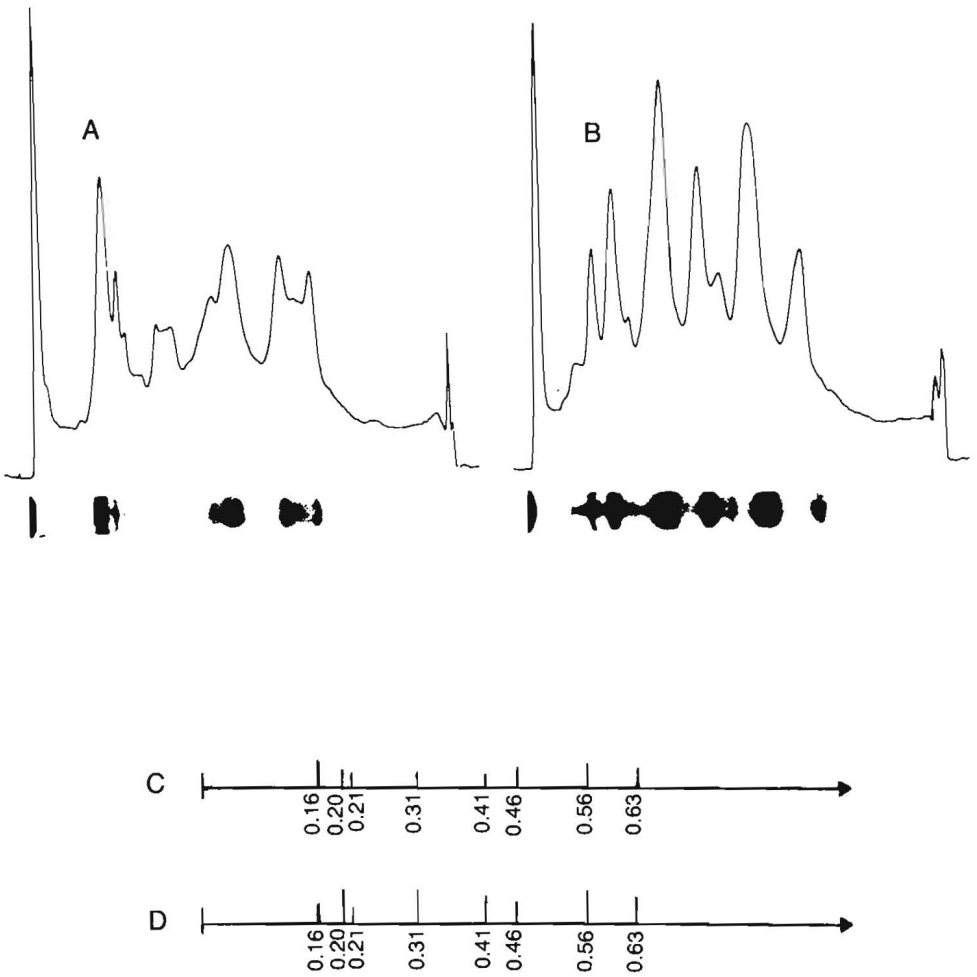

Fig. 2 A-D: a. and b. Photograph of two gels of mature and immature fruiting bodies and their densitometric patterns obtained with a Unicam SP 1800 densitometer.

c. and d. Total protein profile of mature and immature fruiting bodies, respectively. The position of the vertical lines shows the Rp value of the band, and the height indicates the intensity of the stain.

from the left Fig. 2.A) of Rp 0.16 and 0.46, which increased in concentration (Table 3). These findings suggest that during maturation the proteins might be hydrolysed to amino acids, or possibly degraded to low molecular weight proteins. The amino acids derived from protein hydrolysis may provide carbon skeletons for other synthesis in the maturation state, and that may also serve as a nitrogen source for different metabolic pathways. The bands that increased at this stage could be the protein related to the autolysis mechanism, which initiates a number of biochemical processes. These processes might be spore maturation, asci degradation, odor formation of fruit body etc.

Analysis of sporocarp stages (Table 2) showed the presence of sixteen amino acids in protein hyrolysate. Al-Delaimy (1977), reported the presence of seventeen amino acids, including nine essential amino acids in two varieties of truffles.

Table 2. Amino acid composition, on dry weight basis, expressed as mg/100 g truffles, in different stages of *Phaeangium lefebvrei* pat. sporocarps

Amino acid	Immature sporocarps	Mature sporocarps
Aspartic acid	825	944
Glutamic acid	1499	1926
Threonine	405	500
Serine	291	281
Glycine	323	315
Alanine	303	365
Methionine	179	268
Isoleucine	367	524
Valine	667	608
Leucine	998	1058
Proline	736	782
Tyrosine	1056	1165
Phenylalanine	664	697
Histidine	775	837
Lysine	555	555
Arginine	1079	1288
Total amino acids	10058	12113
Total essential amino acids	5025	6335

Table 3. Concentration and percentage of protein bands in two different stages of *Phaeangium lefebvrei* Pat. sporocarp

Band No.	Immature sporocarps*		Mature sporocarps**	
	Protein conc. µg / 100 µl	%	Protein conc. µg / 100 µl	%
1	11.16	6.74	17.26	17.97
2	20.56	12.41	7.60	7.91
3	4.70	2.80	4.01	4.17
4	41.13	24.80	9.88	10.29
5	21.86	13.20	10.09	10.51
6	11.98	7.20	22.26	23.10
7	35.84	21.60	14.87	15.49
8	18.33	11.10	9.99	10.40
Total	165.56	99.85	95.96	99.66

* 100 µl protein sample (eq. 165.6 µg protein) was used. Total soluble protein gained after electrophoretic sep. & staining is 165.56 µg / 100 µl (recovery 99.85%).

** Protein applied 96 µg / 100 µl (recovery 99.66 %).. as indicated above.

Total amounts of the essential amino acids, in the two stages of truffle, comprised about 49.9% and 52.3%, respectively, of the total amino acids detected. The most predominant amino acid present was glutamic acid 14.9% and 15.9% in immature and mature sporocarps respectively. The amount of methionine was 1.8% and 2.2% in immature and mature sporocarps. These results are in agreement with those of Ahmed *et al.* (1981), who reported a glutamic acid content of 15.47% and a combined quantity of cystine and methionine of 4.18% in the Libyan truffle "*Terfezia boundieri*". It is generally found that sporocarp stages have a fairly uniform composition of amino acids within their proteins (Table 2). Notable exceptions to this, however, are the mature stage proteins that are characteristically rich in glutamate and in some instances arginine and leucine. *Phaeangium lefebvrei* had more amino acids than the other truffle studied: *Terfezia claveryi*, *Tirmania nivea* and *T.pinoyi*.

These results may indicate that this type of Iraqi truffle could supply reasonable amounts of protein of high nutritional value if consumed as a food source.

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(Received 17/09/1986;
in revised form 18/08/1987)

التغيرات البايوكيميائية أثناء نضوج الجسم الثمري للفطر *Phaeangium lefebvrei* Pat.

جوليت اوشانا ايواز و [1]منهل مصطفى النعمه

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يعتبر نوع *Phaeangium lefebvrei* Pat. واحد من بين أنواع عديدة من الكمأ الموجود في العراق وأقطار عربية أخرى، إذ يعرف على النطاق المحلي باسم «هوبر». وقد تم خلال هذه الدراسة وصف هذا النوع حياتياً وبعض من الملامح الكيميائية لأول مرة في العراق.

تم استخدام تقنية النقل الكهربي في وسط من هلام أكريل الأميد المتعدد لغرض فصل أنماط البروتينات الكلية الذائبة عند المراحل المختلفة لنضوج الجسم الثمري لهذا الفطر، وظهر بأن أنماط البروتينات الكلية لمراحل نضوج الجسم الثمري تختلف من الناحية الكمية، رغم وجود بعض أوجه التشابه بالنسبة إلى عدد الحزم ومواقع الذروات.

وقد بين الجسم الثمري غير الناضج محتوى عالي من البروتين مشيراً إلى متطلبات أيض في تكوين الأكياس السبورية والسبورات، كما أظهر التحليل بأن الجسم الثمري يمكن أن يعتبر مصدراً غذائياً جيداً من الناحية البروتينية.