The Libyan Journal of Agriculture: volume 10, 1981

Libyan Truffles: Chemical Composition and Toxicity

AHMED ASHOUR AHMED, MOSTAFA A. MOHAMED
AND M. A. HAMI[1]

ABSTRACT

Representative samples of wild truffles—*Terfezia boudieri Chatin*—obtained from the local market in Libya were analysed. The analysis showed that tubers could be considered nutritionally a good source for protein, iron, potassium, calcium and sodium. The results indicated that truffle protein was of high quality, since it contained 9 essential amino acids. They totalled to about 6% of dry truffles. Toxic compounds of the following groups were found to be absent: anthraquinone; cardiac glycosides; cathartic resin and cyanogenetic glucosides.

INTRODUCTION

Truffles, a collective name for the species belong to family (*Tubraceae*), order (*Tuberales*), series (*Discomycetes*), and class (*Ascomycetes*). Four species are common around the world. These are *Terfezia boudieri Chatin*, *Terfezia clavereyi Chatin*, *Terfezia leonis Tul*, and *Terfezia metaxasi chatin* (2,13,16).

Several members of the genus *Tuber* are cultivated over large areas in Italy and France and sizeable quantities of the wild *Terfezia* species are collected and marketed in southern Europe, parts of North Africa and other countries bordering the Mediterranean (13).

The edible portion of the *Terfezia* plant is the receptaculum of a size varying from that of a walnut to a large apple. Surface-colour ranges from light shades of chocolate, to chestnut black. The interior is whitish and acquires a purple tinge near maturity. The consistency of the 'body' is fleshy when mature but is rather hard earlier. The taste and odour are strong, more pronounced after drying, yet agreeable (2,13).

In Libyan Jamahiriya, *Terfezia* grow particularly in the southern and western regions of the country. The tubers are cultivated by hand and marketed. Two tuber species are identified in such areas: *Terfezia boudieri Chatin* (most common) and *Terfezia clavereyi Chatin* (less common)[2]. The growth of such fungus is related to the early rainfall in autumn.

Although *Terfezia* tubers provide large quantities of an apparently rich and agreeable vegetable in the menu of some countries, particularly during early spring when other vegetables are still in the fields, little information is available about their chemical composition and nutritional status. Gray (4) devoted special attention to the utilization of truffles and recorded all known facts about their dietary uses and the extent of their contribution in the menus of some communities. An effort was made by Al-Delaimy (1) who reported the protein and amino acids composition of two varieties of *Terfezia* from Iraq.

[1]*Department of Food Science, Faculty of Agriculture, University of Al-Fateh, Tripoli, S.P.L.A.J.*
[2]*Botany Department, Faculty of Science, University of Al-Fateh, Tripoli, S.P.L.A.J.*

The present study was aimed at presenting some knowledge about the chemical composition and nutritional status of the truffles grown in Libya. Since this plant belongs to the class of fungi, it was thought useful to examine it for the presence, or otherwise, of possible toxic compounds usually occurring in plants, and for specific mycotoxins.

MATERIALS AND METHODS

Sampling

A 10 kg sample of *Terfezia boudieri Chatin* was purchased from the Tripoli market. It had been harvested from the southern hilly tract. Representative portions of *Terfezia* were prepared by cutting it into slices and drying under vacuum at 65°C. The dried material was ground and stored in a desiccator for further analysis.

Methods of analysis: The following determinations were run in triplicate and the results were reported as a mean of the three runs: Moisture content at 65°C under vacuum for 72 hours (3,6), crude fat-soxhlet extraction with diethyl ether (6), crude fibre (3), total carbonated ash, water soluble and insoluble ash (3), Na, K, and Ca content-flame photometer 'Carl Zeiss PF5' (6,9), Fe, Cu, Mn, and Zn-Atomic spectrophotometer 'Perkin–Elmer 360' (6,9), alcohol insoluble solids—AIS—(3), starch (6,7), reducing and non-reducing sugars (3,10).

In the sample under study, the classes of lipids were quantitatively determined. The total lipids were determined using a modified Roese-Cottlab method (3,6). Phospholipids—PL—were quantitatively determined employing the modified Hirsch and Ahrens silicic acid chromatographic column (5). Neutral lipids—NL—and Free Fatty Acids—FFA—were determined using the McCarthey and Duthic alkaline silicic acid chromatographic column (8).

The protein content of our sample was hydrolyzed with 6 N HCl in sealed evacuated glass tubes at 110°C for 24 hours (3,6,9,10). Amino acids were determined in the hydrolyzates by an automatic amino acid analyzer—Beckman Model 121; using the standard procedure (6,10,12).

Various toxic groups were tested by qualitative methods described by Stewart and Stolman (14) and the Official Methods of the A.O.A.C. (3) as follows:

anthraquinone compounds—modified Borntrager test
cardiac glycosides —Baljet's test
cathartic resin —by Mandlin's reagent
cyanogenetic glucosides —sodium picrate paper technique.

This possible occurrence of any compounds resembling in effect of aflatoxins was examined by the chicken-embryo bio-assay method as adopted by the A.O.A.C. (3). The experimental details of the test are as follows:

Ten g. of the dried sample was ground and macerated with 4 successive portions of warm (40–50°C) absolute ethanol and filtered. The filtrate was concentrated to 10 ml by vacuum evaporation and 0.05 ml of this extract was inoculated in each of 30 eggs marked as 'sample'. Aflatoxin B1 solution was standardized to contain 10 ul/ml in ethanol and 0.05 ml of this were inoculated for comparison in each of 30 eggs marked 'aflatoxin'. The control consisted of a set of 30 eggs, each inoculated with 0.05 ml of absolute ethanol.

The development of embryos was watched every alternate day from the 4th day after the incubation was stored till the 18th day when all the eggs were removed from the incubator/shaker and transferred to separate hatching trays for each group.

The hatched chicks were counted and their condition examined after a total of 20 days incubation.

Table 1 Gross chemical composition of Libyan truffles (*Boudieri chatin*)

Constituent	Amount %[a]
	(Mean ± SD)
Moisture	77.70 ± 0.173
Crude fat	6.40[a] ± 0.087
Crude protein	17.19[a] ± 0.053
Carbohydrates	59.73[b]
Crude fibre	3.80[a] ± 0.020
Ash	12.88[a] ± 0.107

[a] On moisture-free basis
[b] Estimated by difference

RESULTS AND DISCUSSION

The average composition of Libyan truffles (*T. boudieri Chatin*) is presented in Table 1. The high value of 77.7% for moisture may decrease the storage period and lower the keeping quality of such a product. The protein content, which averaged 17.19%, appeared to be significantly higher than most vegetables (15). Truffle samples showed a relatively high content of ash (12.88%), as compared to other foods of plant origin (6,15). This may be attributed to the fact that truffles grow their edible parts hypogeously, so large amounts of soil sand might be transferred through them. The crude fibres content of tested samples amounted to 3.80% on dry basis. They are mainly a mixture of cellulosic materials—cellulose and legnin; these are indigestible in the human organism (6,9,10). The relative low content of fibres in Libyan truffles indicated their suitability for direct consumption when considering the nutritional aspects.

Table 2 presents the analysis of some constituents of Libyan truffles. The alcohol insoluble matters—AIM—of truffle samples amounted to 81.75%. The starch content, which averaged 29.02% was comparatively lower than that found in other tubers (6,10,15). 'AIM' has been widely used as a measure of the polysaccarides gums, mucilages, pectins, dextrins, starch and related polyuronides (6,9,10). The alcohol precipitate obtained may also be contaminated with araban, galactan or xylan (6). Data given in Table 2 indicated that about 85% of the ash content of truffles was insoluble in water. In this connection, special attention should be given when preparing truffles for direct consumption.

Table 2 Chemical analysis of different constituents of Libyan truffles (*Boudieri chatin*)[a]

Constituent	Amount %
	(Mean ± SD)
Alcohol insoluble matters	81.75 ± 0.170
Water soluble ash	2.00 ± 0.026
Water insoluble ash	10.88 ± 0.111
Starch	29.02 ± 0.062
Reducing sugars (as maltose)	3.02 ± 0.150
Non-reducing sugars (as sucrose)	0.57 ± 0.190
Neutral lipids	3.02 ± 0.450
Phospholipids	1.81 ± 0.120
Free fatty acids	0.15 ± 0.079

[a] On moisture-free basis.

Table 3 Amino acids composition of Libyan truffles (*boudieri chatin*)[a]

Amino acid	Amount mg/100 g truffles
Alanine	1110.0
Arginine	441.1
Aspartic acid	1564.0
Cystine	284.0
Glutamic acid	2245.4
Glycine	765.5
Histidine	335.3
Isoleucine	748.5
Leucine	1107.3
Lysine	557.7
Methionine	332.4
Phenyl alanine	674.9
Proline	983.5
Serine	943.5
Threonine	1098.0
Tyrosine	495.5
Valine	838.6
Total amino acids	14515.7
Total essential amino acids	6123.8

[a] On moisture-free basis

The fractionation on silicic acid columns was used in separation of lipid classes of Libyan truffles. The neutral lipids, which consist of hydrocarbons, glycerides, sterols and sterol esters, amounted to about 47% of the crude fats of truffles. Phospholipids and free fatty acids constituted about 28% and 2% of the crude fat content, respectively.

The amino acids composition of the truffles under study is presented in Table 3. Seventeen amino acids could be detected in the sample investigated. All the essential amino acids, with the exception of tryptophan which was not analyzed; were found to be present in fair amounts. Total amounts of the essential amino acids comprised about 42.19% of the total amino acids detected. The most predominant amino acids present in the tested samples were glutamic acid (15.47%) and aspartic acid (10.77%). The combined quantity of cystine and methionine in Libyan truffles (4.18% of the total amino acids) provides a reasonable amount of sulfur-containing amino acids. These results are in agreement with the findings reported by Al-Delaimy (1) who found a protein content of 18.8% and 16.2%, on dry basis, in white (*T. hafizi*) and dark (*T. claveryi*) varieties of *Terfezia*, respectively. The white variety was found to contain a concentration of essential amino acids of 6.7%—dry basis, while the essential amino acids in dark variety amounted to 5.1%—dry basis (1). Al-Delaimy (1) established that, in both varieties of truffles, glutamic acid was present in the highest amount. It is worthy of noting that both protein and amino acids content of Libyan truffles studied were found to comprise mostly one-fourth or higher of those present in beef (11). The favourable results obtained may give an evidence that Libyan truffles could supply reasonable amounts of essential amino acids when considered for edible purpose.

It is obvious that mineral elements are of a great nutritional and technological significance when studying foods. Table 4 shows the analysis of macroelements Na, K, Ca, and microelements Fe, Zn, Cu, Mn of Libyan truffles under study. Among the mineral components, potassium was present in the highest concentration. Its concentration (996 mg/100 g) was comparatively higher than that found in other similar foods such as mushroom and potatoes (15). As the microelements are concerned, Libyan truffles considered to be a good source of Fe and Zn.

Table 4 Macroelements and microelements content of Libyan truffles (*boudieri chatin*)[a]

Element	Concentration mg/100 g
Na	29.0
K	996.0
Ca	68.0
Fe	17.0
Cu	8.3
Zn	13.0
Mn	2.2

[a] On moisture-free basis

Table 5 The outcome of chicken-embryo test with truffles extract for qualitative test of toxic compounds in Libyan truffles (*boudieri chatin*).

		Embryos					
	No. of eggs	Failed to develop	Eggs broken in handling with developing embryos	Malformed	Healthy	Total dead embryos + malformed chicks	Total healthy chicks + healthy embryos
Control	28	1	2	Nil	25	1	27
Sample	30	4	Nil	Nil	26	4	26
Aflatoxin	29	25	Nil	4	Nil	29	Nil

It is helpful with many foods belonging to the class of fungi to examine the presence of any possible toxic compounds. In the present study the possible occurrence of some compounds resembling in effects of aflatoxins was examined by the chicken-embryo bio-assay method. Table 5 shows the results of the chicken-embryo test for the presence of aflatoxin-like compounds. All the qualitative tests for the presence of anthraquinone compounds, cyanogenetic glucosides, cardiac glycosides and cathartic resins were negative. It is concluded, therefore, that the variety of truffles under examination, *Terfezia boudieri Chatin*, did not contain any of the toxic substances of the groups mentioned above. In other words the toxic compounds could not be detected at least by the procedure applied for their testing.

Further studies on the vitamin content, the essential fatty-acids content, and the possibility of processing Libyan truffles are in progress.

ACKNOWLEDGEMENT

The authors wish to record their gratitude to Mrs Anka Gardeva for her assistance in the analytical work and the Manager of the University Farm, Mr Mustafa Hudah for his co-operation in arranging the chicken-embryo assay. Also the authors are grateful to the chemists in the Soil and Water Science Department for their assistance in the analysis of microelements.

LITERATURE CITED

1. Al-Delaimy, K. S. 1977. Protein and amino acid composition of Truffles. Can. Inst. Food. Sci. and Tech. J. 10(3): 221–22.

2. Ainsworth, G. C. 1971. Dictionary of the Fungi. 6th ed. Commonwealth Mycological Institute, Kew, Surrey, England.
3. Association of Official Analytical Chemists. 1975. Official Methods of Analysis. 12th ed. Washington, U.S.A.
4. Gray, W. D. 1970. The Use of Fungi As Food and In Food Processing. Butterworths and Co., Publishing Ltd., London.
5. Hirsch, J. and E. M. Ahrens. 1958. The separation of complex lipid mixtures by the use of silicic acid chromatography. J. Biol. Chem. 233: 311–320.
6. Joslyn, M. A. 1970. Methods in Food Analysis. Academic Press, New York.
7. Less, R. 1971. Laboratory Handbook of Methods of Food Analysis (2nd ed.). Leonard Hill, London.
8. McCarthy, R. D. and A. H. Duthic. 1972. A rapid qualitative method for the separation of free fatty acids from other lipids. J. Lipid Res. 3: 117–119.
9. Pearson, D. 1973. Laboratory Techniques in Food Analysis, London Butterworths.
10. Pomeranz, Y. and C. Meloan. 1971. Food Analysis. Theory and Practice. The AVI Pub. Co., Inc., London.
11. Price, J. F. and B. S. Schweigert. 1971. The Science of Meat and Meat Products. W. H. Freeman and Co., San Francisco. U.S.A.
12. Schran, E. J. P., Dustin, S. Moore and E. J. Bigwood. 1953. Application of ion exchange in separation of amino acids of foods. Anal. Chem. Acta 9: 149–162.
13. Signer, R. 1961. Mushrooms and Truffles. Leonard Hill, London.
14. Steward, C. P. and A. Stolman. 1961. Toxicology (11). Academic Press, London.
15. Watt, B. K. and Merrill A. L. 1975. Composition of foods (Agricultural Handbook No. 8). Agriculture Research Science Department of Agriculture, Washington D.C., U.S.A.
16. Winburne, J. N. 1962. A Dictionary of Agriculture and Allied Technology. Michigan State University Press, U.S.A.

الترفاس الليبي
تركيبه الكيماوى وسيمته

د. أحمد عاشور أحمد
د. مصطفى عبد المنعم محمد
محمد أحمد حامي

المستخلص

تم تحليل عينات ممثله من الترفاس النامى فى ليبيا و المتحصل عليه من السوق المحلى بطرابلس . أثبت التحليل احتواء الترفاس على نسبة عاليه من البروتين ـ الحديد البوتاسيوم ـ الكالسيوم ـ و الصوديوم . ولقد دلت نتائج التحليل على أن بروتين الترفاس ذات قيمه غذائية عاليه حيث يحتوى على تسعة أحماض أمنيه أساسيه تصل نسبتها الاجمالية الى ٦ ٪ من الوزن الجاف للترفاس .

تم فى هذا البحث أيضا دراسة امكانية احتواء الترفاس النامى فى ليبيا على أية مواد سامه من نوع افلاتوكسين . ولقد أثبتت النتائج المتحصل عليها خلو الترفاس من أى من المواد السامة المذكورة .