Libyan Truffles: Spoilage, Preservation Fatty Acid and Vitamin Content

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ABSTRACT

Representative samples of wild truffles *Terfezia boudieri* harvested from the southern hilly tract of Libya were studied. The objective was the study of spoilage, preservation, and some nutritional aspects of fresh and preserved truffles. Results have indicated that sterilization is more effective in controlling spoilage organisms than dehydration and freezing. Nutritionally truffles can be considered as a good source of essential fatty acids, vitamin A, B_1, B_2 and niacin.

INTRODUCTION

Truffles are the curious and delicious subterranean fruit of the fungi belonging to the family Tuberceae, order Tuberales, series Discomycetes, class Ascomycetes. Three genera are common around the world, namely *Terfezia, Tirmania* and *Tuber.* The genus Terfezia consist of several species of which two are common in Libya. These are *Terfezia boudieri* and *T. claveryi* (14, 15). Truffles have been used for a long time by Arab bedouins as a meat substitute. Now, truffles are used for their super taste as well as for their high nutritional value. Limited information is available concerning the storage life and the type of microorganisms associated with spoilage of truffles. Al-Delaimy *et al* (2) in their study of micro organisms responsible for spoilage of two types of Iraqi truffles, namely Zbaidy and Harqa isolated the molds: *Rhizopus nigricans, Aspergillus glaucus* and A. repens at storage temperature of 2, 10 and 20-25 °C. A yeast fungus: *Saccharomyees trichothecium* was found on truffles stored at 10C° and at room temperature. *Escherichia coli* and unidentified species of the genus Bacillus were isolated from truffles stored at room temperature (2).

Concerning the nutritive value, Singer (14) and Al-Delaimy *et al.,* (2) have indicated that both French and Iraqi truffles are of high nutritive value with comparison to the other vegetables and tubers. Libyan truffles *(Terfezia boudieri)* contain approximately 17% protein, 6.5% fat, 4% crude fiber, 13% ash and 60% carbohydrates on moisture free basis (1). They also reported that the protein of Libyan truffles is of high

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quality since it contains the most common amino acids, of which nine are essential. Sawaya *et al.*, (12) have indicated that Saudi Arabian truffles contain all the essential amino acids in fairly good concentrations. The mineral content of Libyan truffles was also studied by Ahmed *et al.*, (1). Their results showed that potassium was present in the highest concentration (996 mg/ 100 gm) which is comparatively higher than that found in other foods such as mushrooms and potatoes. The main objective of this work was to study the vitamin and the fatty acid content as well as the preservation of *Terfezia boudieri.*

MATERIALS AND METHODS

Sample collection and preparation:

Fresh samples (40 kgs) *Terfezia boudieri* from the Southern hilly tract of Libya were used. On arrival, the truffles were cleaned to remove the adhering sand. Then the truffles were then washed and peeled. The tubers were then immersed in a mixture of 0.5% ascorbic acid and 0.5% acetic acid to prevent discoloration before being sliced. The sliced bulk was divided into three equal batches.

The first batch was subjected to thermal processing, in which the sliced tubers were blanched for 5 minutes in a mixture of 2% NaCl, 0.5% ascorbic acid and 0.5% citric acid solution. The blanched tubers were then further divided into three portions. Each portion was processed in 1/2 kg glass jar in either 2% NaCl solution or 2% $NaHSO_4$ solution or a mixture of 0.5% ascorbic acid and 0.5% citric acid solution. The jars were exposed for exhaustion at 100°C for 7 minutes before sealing, loaded in the retort and the steam was admitted for a period of 7-10 minutes to bring temperature and pressure to the required levels. Thermal processing was carried out at 121°C for 25 minutes. The jars were cooled in air after being labeled and stored at room temperature for 6 months.

The second batch after being blanched (as above) was drained, and immersed in 2% $NaHSO_4$ solution for 5 minutes. The truffles were then air-dried in a cross dryer after loading the trays. Pre-drying was carried at 60°C for the first 4 hours followed by another 15 hours at 40°C. The dried truffles were placed in polyethylene bags, sealed under vacuum and stored at room temperature for 6 months.

The third batch was blanched in a solution of 0.5% citric acid. The sliced tubers were then immersed in 2% $NaHSO_4$ solution for 5 minutes before sacked under vacuum in polyethylene bags. After sealing, the truffles were slowly freezed in an air flow of approximately 50 feet/ minute at-20C and held at that temperature for 6 months.

Microbial Examination:

The appropriate procedures for examination and identification of the microorganisms responsible for the spoilage of fresh and preserved truffles were applied (5, 7). Plate count agar (PCA) was used for the total plate count and spore forming bacteria, and acidified potato dextrose agar (APDA) for the growth of yeasts and molds. Further identification and confirmation tests of individual organisms were carried out using the compound microscope, gram staining and biochemical techniques (7).

Chemical Examination:

Vitamins determination:

Vitamins were determined according to AOAC, (3) procedures: Vitamin A was determined using a spectrophotometric method (Spectronic 20), (Baush and Lomb USA) Vitamin B_1 and B_2 were determined by fluorometric method (Beckman Ratio, Fluorometer, Germany). Vitamin C was determined using a volumetric method, and Niacin using a colorimetric method (Unicam Sp-600 series 2 spectrophotometer, England).

Lipid extraction and fatty acid composition:

Lipids from fresh, dehydrated, frozen and canned truffles samples were extracted as described by Floch *et al.*, (6) using two extractions with chloroform/ methonol (2: 1, v/ v) in amounts of 10 ml/g tissue. The combined filtered extract of each sample was concentrated to dryness under a stream of nitrogen at 40°C. The fatty acids were determined after transmethylation according to Metcalfe *et al.*, (9) The analysis was carried out with a Pye-Unicam 104 Gas Chromatograph equipped with flame ionizatin detector. The free fatty acids were separated on a 250 × 0.5 (i. d) glass column packed with 8% polyethylene glycol adipate (PEGA) held isothermally at a column temperature of 230°C, and a nitrogen carrier gas flow rate, of 20 ml/ minute. Identification of the peaks was established by comparison of retention time with known standards.

RESULTS AND DISCUSSION

Microbial Examination:

Data on the microbiological examination of fresh, dehydrated, frozen and sterilized truffles are given in Table 1. Results on fresh truffles indicated a high total bacterial count. Count for spore forming bacteria was 7.2×10^3 CFU/g while for yeast and molds the count was 1.8×10^4 CFU/ g. The microbial load of the un-peeled truffles was found to be higher than that of the peeled truffles. This variation is due to heavy contamination of the peel itself. If the truffles for different processes were compared, one can observe that sterilization was more effective than dehydration and freezing as far as total plate count is concerned. Growth of yeast and mold was retarded by the application of dehydration and freezing and completely stopped by sterilization.

From the biochemical tests and microscopic examination, the dominant spoilage flora of fresh truffles were mainly yeasts (*Sacharmoycodes, Candida,* and *Rhodotorula,* and *molds (Mucor* and *Penicillium)*. It appears that the large number of microorganisms contaminating truffles is due to their intimate contact with soil during their growth*. Our results indicate a large number of *Bacillus, Rhizopus, Mucor* and *Penicillium* (13). Also Al-Delaimy *et al* (2) reported that *Rhizopus nigricans* and some species of *Bacillus* were the main microorganisms detected in *Zbaidy* (white truffle) and *Harqa* (Brown truffle).

Vitamin Composition:

Table 2 shows the data on vitamin content of *Terfezia boudieri.* Results showed a

* *Since most of these organisms as preniously found in l'Uyan Truffles habitat soil (13).*

Table 1 — Microbial count of fresh, dehydrated, frozen and sterilized Libyan truffles «Terfezia boudieri» (CFU/ gm)

Products	Total plate count	Spore forming	Yeast & Mold
Unpeeled truffles	2.3×10^6	7.2×10^3	1.8×10^4
Peeled truffles	1.8×10^6	3.1×10^2	5.2×10^3
Peel	3.7×10^6	9.1×10^3	3×10^2
Dehydrated	$< 3 \times 10^2$	$< 3 \times 10^2$	$< 3 \times 10^2$
Frozen	$< 3 \times 10^2$	$< 3 \times 10^2$	$< 3 \times 10^2$
Sterilized			
Packed in brine solution	Nil	Nil	Nil
Packed in sodium bisulfate	Nil	Nil	Nil
Packed in 0.5% citric acid and 5% ascorbic acid	Nil	Nil	Nil

Table 2 — Vitamin Composition of Libyan Truffles (Terfezia boudieri (Mg/ 100 gm)*.

Products	Vitamin A	Vitamin B_1	Vitamin B_2	Niacin	Vitamin C
Fresh	1.93 ± 0.02	28.60 ± 0.00	3.10 ± 0.17	16.67 ± 0.01	18.42 ± 0.14
Dehydrated	1.74 ± 0.01	13.00 ± 0.03	0.86 ± 0.17	6.00 ± 0.1	10.85 ± 0.11
Forzen	1.5 ± 0.01	9.10 ± 0.04	0.59 ± 0.07	3.17 ± 0.03	2.71 ± 0.05
Sterilized:					
Packed in brine sol.	0.96 ± 0.01	4.41 ± 0.05	0.2 ± 0.05	2.85 ± 0.03	5.77 ± 0.04
Packed in Sod. bisulphate	0.93 ± 0.00	3.25 ± 0.03	0.23 ± 0.01	2.73 ± 0.03	5.37 ± 0.04
Packed in 0.5% Citric acid and 0.5% Ascorbic acid solution solution.	0.93 ± 0.01	3.90 ± 0.07	0.24 ± 0.01	2.83 ± 0.01	166.90 ± 0.43

* Mean ± S.D.

higher content of A, B_1, B_2 niacin and vitamin C.

Vitamin C content of truffle samples was high, particularly in the fresh samples (18.42 mg/100 gm). The amount is comparable to that in both species of Saudi Arabian truffles (12). The vitamin C detected (166.9 ± 0.4 mg/ 100 gm) in truffle samples preserved in 0.5% citric and 0.5% ascorbic acid solution was high as expected due to composition of the preserving solution.

There is a marked loss of all vitamins in the preserved samples which were examined after 6 months of storage. These reductions in vitamin content probably occur during the preparation process (e.g. washing, soaking, sulfiting and blanching), heat treatment and a combination of moisture and temperature during storage.

Blanching seems to accelèrate the rate of loss of water soluble vitamins such as B_1, B_2, C and niacin and enhance the stability of vitamin A in dehydrated truffle samples. This suggests that the stability of vitamin A content is generally believed to be due to the inactivation of peroxidase and lipoxidase enzymes which catalyze the destruction of vitamin A and lipid during dehydration and storage.

Loss in total solids and vitamins content in food has been reported by many workers. Baloch *et al.*, (4) indicated that about 10-30% of total solids are lost due to leaching during blanching of carrots. Meanwhile loss of vitamins during preparation processes varied from 10 to 50% in peaches (8).

The considerable loss of Vitamin B_1 in canned truffle samples preserved in $NaHSO_3$ was expected due to the high pH value (alkaline) compared to that preserved in neutral and acidic pH in our study. These results agree with those of Labouza and Tanenbaum (8) and Salunkhe and Dull (11) who reported that the destruction rate of vitamin B_1 increases as pH value increases.

Vitamin A content of the fresh truffle sample was considerably high, compared to the contents of the vitamin in carrot (10). Only a slight decrease in vitamin A content was recorded in frozen and dehydrated truffle samples, whereas a considerable loss was observed in canned samples. This loss in Vitamin A content is mainly due to oxidation.

Fatty Acid Composition

The influence of processing, freezing, and dehydration on the total fatty acid composition of truffles is given in Table 3. The results reveal that the most predominant fatty acid in fresh truffles is $C_{18:2}$, this was followed in descending order of predominancy by C_{16}, $C_{18:1}$, $C_{18:0}$, $C_{8:0}$, $C_{16:1}$, $C_{13:0}$, $C_{14:0}$ and $C_{12:1}$. The various fatty acids content of the canned samples preserved in brine solution were more or less similar to the fresh truffles. The canned truffles preserved in $NaHSO_3$ revealed no appreciable difference in various fatty acid content. The canned truffles preserved in acetic acid and ascorbic acid agreed well in the $C_{16:0\ iso}$ to $C_{18:0}$ contents but recorded higher results for $C_{12:0}$, $C_{12:0}$, $C_{12:1}$, $C_{14:0}$ and $C_{15:0}$. While that for $C_{13:0}$ was lower than the fresh samples. The dehydrated samples with comparison to fresh samples yielded similar results for $C_{14:0}$, $C_{15:0}$, $C_{16:0}$ iso $C_{16:0}$, and $C_{18:0}$. Fatty acids but recorded higher results for $C_{12:0}$ otherwise there were no appreciable differences in the other fatty acids content.

Despite of the lower contents of lipids in of truffles, the difference is evident among the means of most fatty acids with regards to different treatments applied. The varia-

tion in the fatty acid content in treated truffle samples may be due to the influence of the different preservation processes and storage. The variations are probably due to the extent of lipid oxidation, formation of free radicals, and peroxidation during the different treatments and storage (8).

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Table 3 — Fatty acids composition of the Libyan truffle «Terfezia boudieri» (%)

Fatty Acid	Fresh	Frozen	Dehydrated	Brine sol.	Sterilized in Sodium bi-sulfate	0.5% Citric acid & 0.5% Ascorbic acid solution
$C_{8:0}$	1.82 ± 0.06	4.53 ± 0.01	Nil	4.30 ± 0.15	Nil	Nil
$C_{12:0}$	0.20 ± 0.09	6.62 ± 0.03	1.70 ± 0.01	0.53 ± 0.19	0.21 ± 0.08	0.16 ± 0.01
$C_{12:1}$	0.33 ± 0.22	Nil	Nil	0.74 ± 0.28	Nil	0.03 ± 0.01
$C_{13:0}$	0.64 ± 0.17	Nil	1.01 ± 0.01	1.15 ± 0.04	Nil	0.32 ± 0.01
$C_{14:0}$	0.51 ± 0.02	1.74 ± 0.01	0.34 ± 0.07	0.32 ± 0.03	0.21 ± 0.05	0.22 ± 0.01
$C_{15:0}$	0.02 ± 0.01	1.05 ± 0.01	Nil	Nil	Nil	0.10 ± 0.09
$C_{16:ISO}$	0.15 ± 0.04	3.83 ± 0.01	0.34 ± 0.01	0.94 ± 0.01	Nil	0.03 ± 0.01
$C_{16:0}$	18.72 ± 0.01	33.80 ± 0.01	23.31 ± 0.01	22.98 ± 0.05	17.28 ± 0.01	19.56 ± 0.01
$C_{16:1}$	0.68 ± 0.09	0.35 ± 0.01	Nil	0.74 ± 0.13	0.48 ± 0.13	1.55 ± 0.01
$C_{18:0}$	3.23 ± 0.06	2.44 ± 0.01	2.36 ± 0.03	3.04 ± 0.13	2.18 ± 0.23	3.06 ± 0.21
$C_{18:1}$	17.67 ± 0.01	14.98 ± 0.03	19.19 ± 0.00	11.65 ± 0.48	10.86 ± 0.49	16.83 ± 0.07
$C_{18:2}$	56.03 ± 0.02	30.66 ± 0.01	51.75 ± 0.01	53.61 ± 0.01	68.78 ± 0.06	58.43 ± 0.02

Mean ± S.D.

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المستخلص

لقد أجريت هذه الدراسة على عينات نموذجية من الترفاس *Terfezia boudieri* الواسع الانتشار في المنطقة الجنوبية المتاخمة للمرتفعات. وكان الهدف من هذه الدراسة هو تحديد أسباب الفساد والتلف الذي يصيب الترفاس وايجاد أحسن طرق الحفظ الملائمة لإطالة الفترة التخزينية له. كما أن الدراسة اشتملت على بعض النواحي الأخرى من حيث دراسة الأحماض الدهنية والفيتامينات ومقارنتها في كل من الترفاس الطازج والمحفوظ.

وقد أظهرت النتائج أن الحفظ بالتعليب هو من أفضل الطرق من حيث الحد من الفساد والتلف الناتج عن فعل الكائنات الحية الدقيقة، مقارنة بالحفظ عن طريق التجفيف أو التجميد. كذلك أوضحت الدراسة بأن الترفاس يعتبر من المصادر الجيدة للأحماض الدهنية الضرورية وكذلك لبعض الفيتامينات مثل فيتامين (أ)، فيتامين ($ب_2$) وفيتامين النياسين.