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# *Research Articles*

# Study of the Lipid Fraction of Freeze-Dried Dandelion Root

By SALAH ELDIN F. ALI† and EARL P. GUTH

**Gas chromatographic studies of the lipid fraction from oven-dried and freeze-dried dandelion root reveal significant difference. Synthesis of fatty acids apparently continues during the oven drying process.**

IT IS WELL known that on harvesting an entire plant or removing parts from the plant, many of the vital processes do not stop immediately (1). It is also known that the length of time and the degree of temperature required for drying plant materials affect the rate and intensity of these processes and, consequently, could affect the nature of some constituents normally present. The chemical reactions which occur in plant cells are apparently accelerated by enzymes. Therefore, as long as conditions are favorable to enzymatic action, these reactions will proceed. The rate at which an enzymatic reaction proceeds is influenced not only by the temperature, but also by the length of time that the reaction mixture has been maintained at that temperature. Within limits, an increase in the

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† College of Pharmacy, University of Baghdad, Baghdad, Iraq.

temperature increases the rate of enzymatic reactions. When the optimum temperature is exceeded, there is a decrease in the rate of these reactions because of a denaturation effect upon the enzyme (2–4).

Since high temperatures do affect some plant materials during the process of their drying, it would be reasonable to dry these materials at low temperatures. The freeze-drying process has proved to be feasible for drying and preserving plant and animal products and the application of this method as a means of drying crude drugs and drug extracts has been employed by many investigators (5–10).

The purpose of this study is to investigate the possibility that certain plant principles which are now known may not necessarily be the actual constituents of that source. In order to achieve this objective, an attempt was made to examine specifically some of the chemical changes which may occur after the plant has been harvested and dried.

This paper is concerned with the differences found in the lipid fraction of dandelion root (*Taraxacum officinale*).

## EXPERIMENTAL

**Collection and Drying.**—The roots of dandelions were gathered at three different seasons; Autumn, Spring, and Summer, from a field adjacent to the Ohio State University.

The roots were gathered and divided in two lots each time; one lot was immediately frozen with dry ice.

The unfrozen roots were cleaned and then dried in an oven at 50°; these were designated as O.D. samples. The frozen roots were rapidly cleaned and freeze-dried at a temperature not higher than 25° and a pressure of 100 to 200 $\mu$ of mercury; these were designated as F.D. samples.

**Determination of Fatty Acids.**—The lipid-containing fraction of the roots was extracted with ether in a Soxhlet apparatus. The ether was then removed by vacuum distillation, the extracts dried in a desiccator, and stored under nitrogen at 0° in a tightly closed container.

**Preparation of the Methyl Esters of the Fatty Acids.**—The dried ether extract was saponified with 10% alcoholic potassium hydroxide. After the nonsaponifiable matter was removed, the soap solution was acidified with 25% sulfuric acid and washed with ether. The ether was removed by vacuum distillation.

The fatty acids so obtained were dissolved in anhydrous ether and methyl esters prepared using diazomethane.

The constituent fatty acids were tested and identified, as their methyl esters, using gas-liquid chromatography.[1] The gas-chromatographic apparatus employed in this study was a Wilkens Areograph model A-110-C.

[1] These findings were confirmed by Mr. Robert B. Iden, Battelle Memorial Institute, Columbus, Ohio.

**Preferred Operating Conditions.**—To minimize the effects of the many operative variables, the following conditions were established: a column packed with 10% diethylene glycol succinate on 60–80 mesh firebrick; column dimension, 72 in. × 0.25 in. (i.d.); flow rate, 90 ml./min. helium; temperature, 185° for the resolution of low molecular weight fatty acid methyl esters and 195 to 198° for the resolution of higher molecular weight fatty acid methyl esters. The linearity of the plot of the logarithm of apparent retention volume ($V_R'$) *vs.* the chain length for a number of known saturated straight-chain fatty acid methyl esters was used as an indication of constant column temperature for each analysis.

**Identification of Fatty Acids.**—The retention volume ($V_R'$) of each component fatty acid was precisely measured to the nearest 0.1 cm.

The preliminary characterization of the fatty acid components of the lipid fraction in each of the oven-dried and freeze-dried root, collected at different seasons, was obtained by simple comparison of their apparent retention volume with those of known saturated fatty acid methyl esters from a standard curve prepared under similar conditions.

The identification of the peaks was further confirmed by the addition of a known synthetic mixture of pure fatty acid methyl esters that correspond to the suspected peaks to the solution of the unknowns, and a new chromatogram was obtained. The results and the relative proportions of the fatty acid methyl esters separated from the lipid fraction of the root are listed in Table I.

TABLE I.—COMPONENT FATTY ACIDS OF LIPID FRACTION OF FREEZE-DRIED AND OVEN-DRIED DANDELION ROOT, SPRING COLLECTION

| Fatty Acid | Chain Length | Relative Proportion, % O.D. | F.D. |
|---|---|---|---|
| Caprylic | $C_8$ | trace | ... |
| Capric | $C_{10}$ | trace | ... |
| Lauric | $C_{12}$ | 0.29 | 0.3 |
| Myristic | $C_{14}$ | 3.94 | 5.1 |
| Unknown 1 | ... | 0.56 | 0.5 |
| Unknown 2 | ... | 0.23 | 0.4 |
| Palmitic | $C_{16}$ | 21.29 | 20.2 |
| Unknown 3 | ... | 0.30 | 0.8 |
| Unknown 4 | ... | 0.28 | 0.3 |
| Stearic | $C_{18}^{5}$ | 1.03 | 1.8 |
| Oleic | $C_{18}^{1=}$ | 3.95 | 4.1 |
| Unknown 5 | ... | 0.00 | 0.2 |
| Linoleic | $C_{18}^{2=}$ | 44.89 | 40.4 |
| Unknown 6 | ... | 1.14 | 1.0 |
| Linolenic | $C_{18}^{3=}$ | 21.42 | 16.7 |
| Unknown 7 | ... | 0.67 | 4.1 |
| Unknown 8 | ... | trace | 4.1 |

## DISCUSSION

The neutralization number of the crude ether extracts of the root (Table II) indicate that there was a significant increase in the saponification number of the ether extract of freeze-dried Summer root as compared with the corresponding oven-dried sample. This would indicate that the concentration of low molecular weight fatty acids in the extracts obtained from the freeze-dried sample was higher than those present in the extracts obtained

TABLE II.—SAPONIFICATION NUMBER OF THE LIPID FRACTION OF THE FREEZE-DRIED AND OVEN-DRIED DANDELION ROOT (U.S.P. XVI)

| Sample | Autumn O.D. | Autumn F.D. | Spring O.D. | Spring F.D. | Summer O.D. | Summer F.D. |
|---|---|---|---|---|---|---|
| 1 | 111.5 | 90.8 | 124.5 | 123.6 | 152.1 | 276.3 |
| 2 | 105.2 | 96.3 | 123.7 | 118.5 | 159.3 | 280.5 |
| 3 | 110.2 | 99.4 | 119.3 | 123.3 | 166.1 | 281.6 |
| Av. | 108.9 | 95.5 | 122.5 | 121.8 | 159.2 | 279.4 |

from the oven-dried sample. Extracts from the oven-dried Autumn and Spring collections showed a slight increase in saponification numbers over those obtained from the freeze-dried samples. Such differences between crude products are not usually considered to be reliable. Therefore, no immediate interpretation was made, the composition and degree of purity of the extracts being unpredictable. A negative iodine number was obtained for the ether extract prepared from the freeze-dried Summer root as compared with the extract prepared from the oven-dried sample. The iodine number of the extracts prepared from the oven-dried Autumn and Spring roots indicated the existence of a relatively higher degree of unsaturation as compared with the corresponding freeze-dried samples.

The distribution of fatty acids (Table III and Fig. 1) found in the freeze-dried and oven-dried root of the Spring collection shows some interesting differences. Myristic and stearic acids appear to be slightly greater in the freeze-dried sample. In addition, unknown fatty acids Nos. 2, 3, and 7 are greater in the freeze-dried sample. Unknown fatty acids Nos. 5 and 8 appear only in the freeze-dried sample. On the other hand, palmitic, linoleic, and linolenic acids appear in greatest quantity in the oven-dried sample.

Myristic, palmitic, and oleic acids appeared to be greatly increased (Fig. 2 and Table IV) in the freeze-

TABLE III.—IODINE NUMBER OF THE LIPID FRACTION OF THE FREEZE-DRIED AND OVEN-DRIED DANDELION ROOT (U.S.P. XVI)

| Sample | Autumn O.D. | Autumn F.D. | Spring O.D. | Spring F.D. | Summer O.D. | Summer F.D. |
|---|---|---|---|---|---|---|
| 1 | 64.5 | 65.9 | 99.7 | 89.5 | 52.2 | ... |
| 2 | 63.1 | 57.0 | 98.2 | 93.5 | 54.1 | ... |
| Av. | 63.8 | 61.5 | 98.9 | 91.5 | 53.2 | ... |

TABLE IV.—RELATIVE DISTRIBUTION OF SOME IMPORTANT FATTY ACIDS OF FREEZE-DRIED AND OVEN-DRIED DANDELION ROOT, AUTUMN COLLECTION

| Fatty Acid | Chain Length | Relative Proportion, % O.D. | Relative Proportion, % F.D. |
|---|---|---|---|
| Myristic | $C_{14}$ | 5.24 | 15.06 |
| Palmitic | $C_{16}$ | 31.66 | 37.63 |
| Oleic | $C_{18}{}^{1=}$ | 3.14 | 4.68 |
| Linoleic | $C_{18}{}^{2=}$ | 47.43 | 33.86 |
| Linolenic | $C_{18}{}^{3=}$ | 12.77 | 8.70 |

dried sample of the Autumn collection. Linoleic and linolenic acids are found to be considerably greater in the oven-dried sample of this collection.

Unknown fatty acid No. 1 (Fig. 3) appeared in greatest quantity in the freeze-dried root of the Summer collection, while it appeared only in a minute quantity in the oven-dried sample. This increase may explain, in part, the reason for a higher saponification number of the freeze-dried Summer sample over that of the corresponding oven-dried samples.

The presence of traces of caprylic and capric acids in the oven-dried sample of the Spring collection is also indicated by a slight increase in the saponification number of the ether extract obtained from the roots that were oven-dried over those of the corresponding freeze-dried sample. The appearance of these two acids in the oven-dried samples, while none appear in the freeze-dried sample, may be due to loss of these fatty acids during the freeze-drying process, since this was performed at a pressure of 200 $\mu$. On the other hand, this difference might represent a breakdown of larger fatty acids due to higher temperature in the oven-drying process.

In view of these findings, it might be reasonable to assume that the decrease in the quantities of myristic acid in the lipid fraction of the oven-dried samples of the Spring and Autumn roots as compared with the freeze-dried samples indicates a continued synthesis of fatty acids in the oven-dried samples. Such process may be blocked by freeze-drying the root. This is also indicated by a considerable decrease in palmitic acid content of the oven-dried Autumn samples as compared with the corresponding freeze-dried samples. The loss in

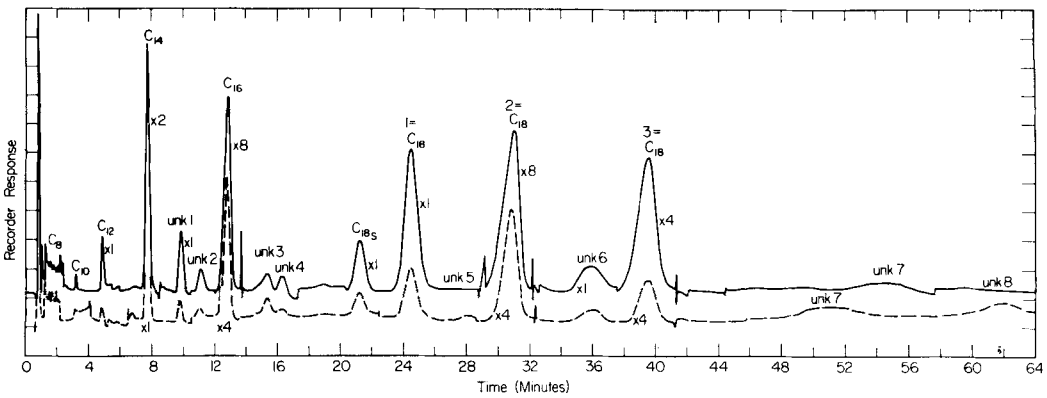


Fig. 1.—Separation of methyl esters of fatty acids in freeze-dried and oven-dried dandelion root at 196°, Spring collection. Samples: —— (B) 4.0 $\mu$l. O.D., – – (A) 5.7 $\mu$l. F.D.; column packing, 10% E.G.S. on firebrick; column, 144 in. × 0.25 in.; flow rate, 105 ml./min.; temp., 196°; current, 236 mv.

stearic acid of the oven-dried samples of the Spring collection and that of oleic acid in the oven-dried samples of the Spring and Autumn collection tends to support this conclusion.

In the oven-drying process, the Spring collected root shows a higher content of palmitic, linoleic, and linolenic acids over the freeze-dried sample, which seems to indicate that synthesis of the fatty acids continues in the oven-drying process. It is further believed that little degradation occurs in the oven-drying process since, if this did occur, there should be less of these larger fatty acids and a greater percentage of smaller fatty acids. The data (Table I) show, however, that there is a greater percentage of small molecular weight fatty acids and a smaller percentage of the larger fatty acids in the freeze-dried sample. This finding also supports the contention that synthesis does continue for a time during the oven-drying process. This is further supported by the fact that these differences are more marked

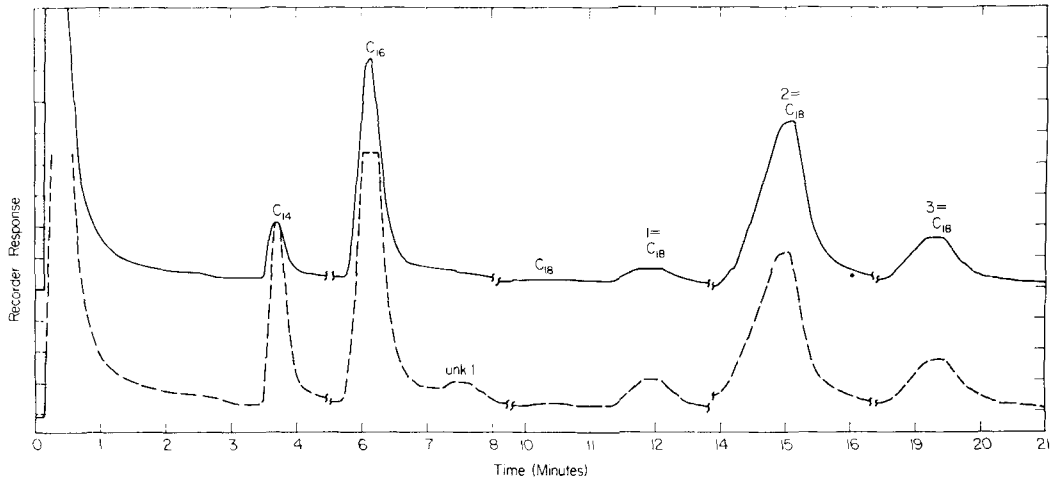


Fig. 2.—Separation of methyl esters of fatty acids in freeze-dried and oven-dried dandelion root at 195°, Autumn collection. Samples: ——(B) 5.0 μl. O.D., – –(A) 5.0 μl. F.D.; column packing, 10% E.G.S. on firebrick; column, 72 in. × 0.25 in.; flow rate, 90 ml./min.; temp., 195°; current, 220 mv.

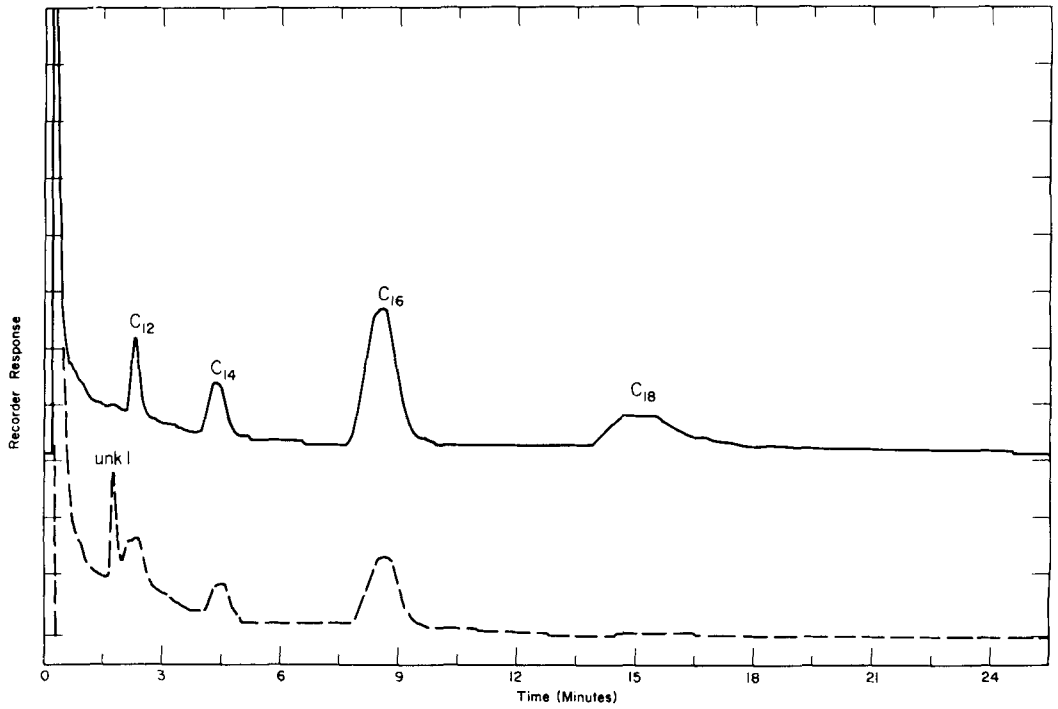


Fig. 3.—Separation of methyl esters of fatty acids in freeze-dried and oven-dried dandelion root at 198°, Summer collection. Samples: ——(B) 8.0 μl. O.D., – –(A) 6.5 μl. F.D.; column packing, silicon on firebrick; column, 72 in. × 0.25 in.; flow rate, 90 ml./min.; temp., 198°; current, 220 mv.

in the Autumn collection which required 6 days to oven-dry as compared with the Spring collection which was dried in 3 days.

Unknown acids Nos. 1, 2, 3, 4, and 6 may possibly be branched-chain, unsaturated, or odd-numbered fatty acids of different chain length. The difference between the quantities of these fatty acids in the oven-dried and freeze-dried samples can also be explained in reference to the amounts of the larger fatty acids present.

The appearance of unknown acids Nos. 5 and 8 only in the freeze-dried samples of the Spring root is of special interest. It was observed that the solutions of methyl esters of fatty acids of the freeze-dried samples were unstable. After a short time, the solutions developed a white precipitate. Consequently, fresh solutions were always used in the gas chromatographic determination of the freeze-dried samples. The solutions of the esters of the oven-dried samples were apparently stable in that they did not show any precipitation over a 12-hr. period. This observation suggests the possibility that unknown acids Nos. 5, 7, and 8 may be highly unsaturated compounds or otherwise unstable molecules which could have been destroyed in the oven-dried samples.

The alteration in the fatty acid distribution, as illustrated by this work, could suggest that other components of this plant are altered by methods used in curing the plant.

## SUMMARY AND CONCLUSIONS

1. The fatty acid contents are greatly affected by the temperature and time required for drying dandelion root.

2. Oven-drying does greatly influence the distribution and molecular nature of fatty acid components of dandelion root as compared to freeze-drying.

3. It appears that myristic and stearic acid contents are higher in the freeze-dried than in the oven-dried dandelion root of the Spring collection. Myristic, palmitic, and oleic acids are higher in the freeze-dried than in the oven-dried root of the Autumn collection.

4. In oven-drying process, the Spring and Autumn collected roots show a higher content of linoleic and linolenic acids over the freeze-dried samples, which seems to indicate that synthesis of fatty acids continues in the oven-drying process.

5. It is believed that little degradation occurs in the oven-drying process since, if this did occur, there should be less of these larger fatty acids and a greater percentage of smaller fatty acids.

6. The presence of a greater percentage of small molecular weight fatty acids and a smaller percentage of the larger fatty acids in the freeze-dried samples suggests that synthesis does continue for a time during the oven-drying process.

7. Unknown fatty acids Nos. 2, 3, and 7 are greater in the freeze-dried root of the Spring collection than in the oven-dried samples. Unknown fatty acids Nos. 5, 7, and 8 appear only in the freeze-dried root.

8. Unknown fatty acids Nos. 1, 2, 3, 4, and 6 may possibly be branched-chain, unsaturated, or odd-numbered fatty acids of different chain lengths.

9. Unknown acids Nos. 5, 7, and 8 may possibly be highly unsaturated compounds.

10. The changes occurring in the fatty acid distribution of the root as a result of the two methods of drying indicate that the method of drying a plant material can have a marked effect on these molecules.

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