Figure 5 and Figure 9 are not directly comparable, on the other hand. Figure 5 shows the birefringence of a particle of plant fibrous cellulose from food which had passed through the human body and was recovered in the stool which was freezer-stored upon evacuation. Figure 9, conversely, is a particle of commercial alpha cellulose which had not been eaten, but which is included to show the characteristic birefringence of fibrous cellulose per se.

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NUTRIENTS IN ALFALFA

Lipids of Dehydrated Alfalfa (*Medicago sativa*)

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Lipids of dehydrated alfalfa (6.4%) were fractionated by silicic acid chromatography. The first fraction, accounting for 30% of the total lipids, contained 30% low molecular fatty acids, mainly C_8. The main glyceride lipids were mono- and digalactosyldiglycerides. The galactolipids contained approximately 80% linolenic acid. Considerable amounts of unsaponifiables were present in all fractions.

Loss of carotene during storage of dehydrated alfalfa is of sufficient magnitude to be responsible for an appreciable decrease in the economic value of the alfalfa. Noncarotene lipid constituents probably play an important role in this loss, and since details of their composition have so far not been completely elucidated, such studies have been initiated in this laboratory. Hilditch (*12*) summarized information on the nature of green leaf lipids. Recent developments are those of Carter *et al.* (*7–9*), who isolated a mono- and a digalactosyldiglyceride from flour lipids; Benson *et al.* (*3*), who showed that the major chloroplast lipids are galactolipids; Lepage *et al.* (*17*), who described a sulfolipid in alfalfa and identified it as a 6-sulfo-6-deoxy-*O*-α-D-glucopyranosyldiglyceride; Weenink (*22*), who demonstrated that the major lipids of the acetone-solubles of red clover are mono- and digalactolipids; and Sastry and Kates (*19*), who isolated and characterized mono- and digalactosyldiglycerides from runner-bean leaves. The purpose of this paper is to report some observations on the nature of the lipids in dehydrated alfalfa meal.

The dehydration of alfalfa is usually accomplished at initial air temperatures above 550° C., although the temperature of individual pieces may vary considerably (*16*). Analysis of the lipids of dehydrated alfalfa might be expected to be complicated by the products of oxidation, degradation, or rearrangement. A comparison of the results obtained in this study with those obtained from fresh alfalfa is in progress.

Material and Methods

Alfalfa meal was obtained immediately after dehydration from the Dixon Dryer Co., Dixon, Calif., transferred to 1-gallon, crimped-top cans, and stored at −18° C. The lipids were isolated from the alfalfa meal by continuous extraction with 95% ethanol for 48 hours in a modified Soxhlet apparatus. The ethanolic solution was dried in vacuo at 45° C. with a flash evaporator (Laboratory Glass and Instrument Co.). The residue was taken up in chloroform; the deep green chloroform solution was washed twice with water and evaporated as before. The residue was a green, viscous material amounting to 6.4% by weight of the original alfalfa. It was again dissolved in chloroform and stored under nitrogen at −18° C., protected from light until used. Efforts to separate phospholipids from the main lipid with cold (0° C.) acetone were unsuccessful.

The lipids were separated into fractions by silicic acid (Mallinckrodt, 100 mesh, analytical grade) chromatography. Chloroform with increasing amounts of methanol was the eluent (*21*). The various fractions were saponified by the method described by James (*15*). The saponified acids were methylated with 2,3-dimethoxypropane (courtesy of The Dow Chemical Co.) (*18*), and the resulting methyl esters were analyzed by gas chromatography. Diethylglycol succinate was used as the liquid substrate, and siliconized (*5*) firebrick (30 to 60 mesh), as the stationary phase.

Table I. Elution of Lipid from Silicic Acid Column

Combined Fractions	Solvent, % Methanol in $CHCl_3$	Solvent, Liters	Material, Grams	Yield, %
2–5	0.5	0.5	1.04	29
6–11	5	0.6	1.02	29
12–15	8	0.3	0.47	13
16–26	12–25	1.1	0.63	18
27–36	50–100	1.1	0.36	10

Table II. Analyses of Alfalfa Lipid Fractions

Material	Unsap., %	Fatty Acid, %	Reducing Sugar as Galactose, %	Fatty Acid–Sugar (Molar Ratio)
Fractions 2–5	42	25	Trace	...
Fractions 6–11	11	35	11	2.00
Fractions 12–15	9	27	18	0.94
Fractions 27–36	21	17	8	1.33
Total extract	16	35	8	...
Monogalactosyldiglyceride[a]	..	67.4	21.1	2.00
Digalactosyldiglyceride[a]	..	55.8	36.5	1.00

[a] Theoretical; based on linolenic acid as the fatty acid.

Table III. Fatty Acid Analyses of Column Fractions[a]

Fatty Acid	Total Extract, %	Fractions 2–5, %	Fractions 6–11, %	Fractions 12–15, %	Free Fatty Acids, %
5:0	..	tr[b]	..	..	..
6:0	tr[b]	tr	..	..	..
8:0	1	28	..	..	..
10:0	tr	2	tr[b]	tr[b]	..
11:0	tr	tr	tr	tr	..
12:0	1	3	tr	tr	..
14:0	2	3	tr	tr	..
16:0	19	9	7	12	19
16:1	3	5	..	tr	2
18:0	1	..	1	2	1
18:1	2	7	tr	tr	1
18:2	14	16	7	2	14
18:3	58	26	85	81	64
20:0	..	..	..	3	..
20:2	..	..	tr	tr	..
22:0	..	..	tr	tr	..

[a] *cf.* Figure 1.
[b] tr indicates amounts less than 0.5%; amounts greater than 0.5% were rounded off to nearest whole per cent.

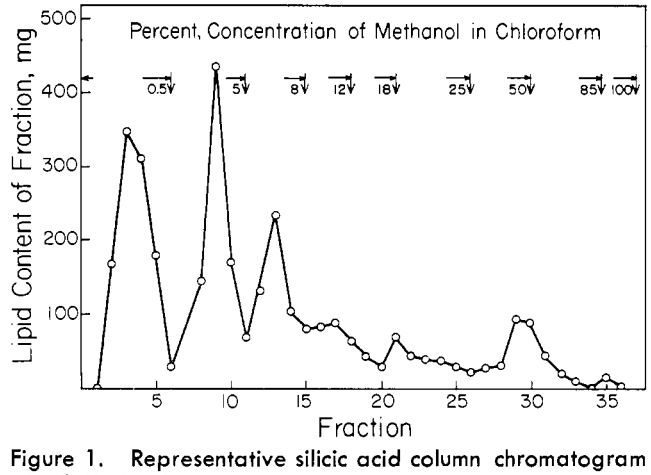

Figure 1. Representative silicic acid column chromatogram of alfalfa lipids

Each fraction was 100 ml.

Aerograph Model A-110-C was used Column temperatures were 184° and 198° C. The peaks were measured by the method of Carroll (*6*). Carbohydrate was determined, with galactose as standard, with anthrone by the method of Bailey (*1*).

Free fatty acids were determined by a resin technique (*13*) and analyzed by gas chromatography.

Results and Discussion

Approximately 72% of the chloroform-soluble fraction of the ethanolic extract was eluted in the first three peaks from the silicic acid column (Figure 1 and Table I). The remaining 28% of the material was eluted slowly and required higher percentages of methanol in the eluent. Separation and analysis of all alfalfa-lipid fractions were hampered by the presence of pigments and their degradation products, especially those of the chlorophylls.

Fatty acid, unsaponifiable, and carbohydrate analyses of the four main fractions gave unexpectedly low fatty acid and high unsaponifiable matter content (Table II), indicating that derivatives of glyceride ethers and waxes were present. Jackson and Kummerow (*14*) reported that alfalfa meal extract contained 17% crude waxes. Blair *et al.* (*4*) showed that the waxes of alfalfa meal consisted of hydrocarbons (70%) and esters (30%). The hydrocarbons could account for high unsaponifiable content in fractions 2 to 5. The esters could account for the high content of unsaponifiables in the later fractions. Shorland (*20*) found that fatty acids accounted for 70 to 75% of the acetone-soluble fraction of the ethanolic extract of fresh grasses.

Thirty per cent of the fatty acids of fractions 2 to 5 occurred as C_8 and C_{10} acids (Table III). Such large amounts of low molecular weight acids have not previously been reported in fresh grasses (*10, 20*).

Fractions 6 to 11 and 12 to 15 (Table III) had a high linolenic acid content. Weenink (*22*) and others (*10, 20*) showed that the fatty acids of the galactolipid fraction of grasses are 80 to 90% linolenic acid.

The fatty acid–sugar molar ratios of these fractions were compared with the theoretical values for a monogalactosyldiglyceride and a digalactosyldiglyceride (Table II). The values given in Table II together with the fatty acid analysis (Table III) indicate that fractions 6 to 11 may be largely monogalactosyldiglyceride, and fractions 12 to 15 may be largely digalactosyldiglyceride. However, the concurrence may be fortuitous.

The infrared spectra of fractions 27 to 35 (Figure 1, Table I) were almost identical with the infrared spectra of lysolecithin isolated from yeast by Hanahan *et al.*

(*11*). However, the fractions contained a sugar moiety. Fractions 27 to 35 were possibly a mixture of lysolecithin and a sugar lysolipid–e.g., lysosulfolipid. Yagi and Benson (*23*) have shown that the diglyceride sulfolipid of Benson *et al.* (*2*) can be converted enzymatically to a lysosulfolipid.

The free fatty acids, which amounted to 7% of the total lipid extract, contained relatively more palmitic and linolenic acids and less stearic and oleic acids than Garton (*10*) found using fresh pasture grasses. The high percentage of linolenic acid in the free fatty acids is to be expected since the total lipids have a similar fatty acid distribution (Table III).

The process of dehydrating alfalfa is such that oxidation and degradation of the lipids could well occur. Possibly the high content of C_8 and C_{10} acids, free fatty acids, and the unsaponifiable fraction are at least in part a reflection of changes occurring during the dehydration process. Studies now underway will compare the lipids of fresh alfalfa with those found in the dehydrated meal.

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MILK FAT ANALYSIS

The Protection of Milk Fat Tocopherols during Saponification with Ascorbic Acid

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Difficulties encountered in attempting to determine the total tocopherols on the unsaponifiable matter of milk fat were overcome through the use of ascorbic acid in the saponification mixture. An analytical procedure utilizing this step in the chemical determination of tocopherols, that enables one to determine concurrently and with high degree of precision vitamins A and E and carotenoids on the unsaponifiable matter of fat, permitting the analysis of six samples in one day, was evolved. Information on the protective influence which ascorbic acid extends to tocopherols in alkaline medium is of value in connection with studies of organoleptic and nutritional properties of lipid-containing food products processed with ionizing energy, and of metabolic processes involving vitamin E.

Moore and Tosic (*7*) reported, on the basis of recovery tests, that the loss in *d,α*-tocopheryl acetate during saponification may be completely prevented by a suitable procedure for adding pyrogallol before saponification. They remarked that the need for saponification in the determination of tocopherols in lipids results from the fact that the Emmerie and Engel (*3*) reaction is not given by tocopheryl acetate, and thus vitamin E present in esterified form in natural sources might escape estimation if saponification is omitted.

Quaife and Harris (*11*), in their paper on molecular distillation as a step in the chemical estimation of tocopherols, indicated that saponification, which would appear to be a proper procedure for concentrating tocopherols from lipids, has not proved satisfactory for fats of low tocopherol content, even if conducted in a closed system according to directions given by Chipault *et al.* (*1*) with the use of pyrogallol as an antioxidant.

More recently, Handwerk and Bird (*4*) evolved a procedure for the determination of unoxidized tocopherols in milk fat based on the Moore and Tosic saponification step to protect vitamin E