TABLE I

Spray Reagent Characteristics of Human Lenticular Lipid Classes

Spot No. [a]	Reagent: Char [b]	PLS [c]	Nin [d]	α-Nap [e]	Lipid classes
1	+	−	−	+	Neutral lipids (cholesterol and glycerides)
2	+	−	−	−	Free fatty acid
3	+	+	+	+	Phosphatidyl ethanolamine and glycolipid
4	+	−	−	+	Glycolipid
5	+	−	−	+	Glycolipid
6	+	+	−	−	Phosphatidic acid
7	+	+	+	−	Phosphatidyl serine
8	+	+	−	−	Phosphatidyl inositol
9	+	+	−	−	Phosphatidyl choline
10	+	+	−	−	Sphingomyelin
11	+	−	+	+	Glycolipid
12	+	−	+	+	Glycolipid
13	+	−	+	+	Glycolipid

[a] Numbers refer to spots in Figure 1.
[b] 55% sulfuric acid plus 0.6% potassium dichromate reagent (Rouser et al. JAOCS *41*, 836–840 (1964).
[c] Specific for phospholipids (Dittmer and Lester, J. Lipid Res. *5*, 126–127 (1964).
[d] Ninhydrin reagent (0.1% in *n-butanol*). Color developed by heating for 3–5 min at 120C.
[e] Specific glycolipid spray of 0.2% α-naphthol in ethanol followed by a light spray with 95% H_2SO_4 and heating at 120C. (Siakotos and Rouser, unpublished). Cholesterol gives a color.

Bovine lens was found to contain similar components. It is interesting that sphingolipids are the predominant polar lipid components in lens of man and cattle and it appears that sphingolipids have special significance in the structure and function of the lens. It seems probable that these lipids are largely components of membrane structures.

GERALD L. FELDMAN
LUTRELL S. FELDMAN
Department of Ophthalmology
Baylor University College of Medicine
Houston, Texas
and
GEORGE ROUSER
Department of Biochemistry
City of Hope Medical Center
Duarte, California

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Reduction of Hydroperoxide Interference in the 2,4-DNP Determination of Carbonyls

The 2,4-dinitrophenyl hydrazine (2,4-DNPH) method of Henick et al. (1) for the determination of carbonyl in fats has been widely used because it is relatively simple and quite sensitive. These positive attributes are offset by the fact that the strong acid used (trichloroacetic) and the high temperature (60C) cause the decomposition of hydroperoxides and the formation of additional carbonyls.

This fact has been reported in the literature (2, 3) and has been verified in this laboratory in our work on autoxidized and thermally oxidized oils. Hence, reduction of the hydroperoxides in the oils with $NaHSO_3$ or HI has given substantially lower results. Reduction, however, does not seem to be a complete solution to the problem for, besides being time-consuming, it generates additional carbonyls (4).

TABLE I

Decomposition of *t*-Butyl Hyproperoxide [a] by Trichloroacetic Acid

Reaction conditions: Time	Temperature	Optical density of basic 2,4-DNP's formed: 430 mμ	460 mμ
30 min	60C	0.345	0.317
2 hr	23C	0.118	0.073
20 hr	5C	0.115	0.090
7 days	−18C	0.081	0.068

[a] Concentration of *t*-Butyl Hydroperoxide = 6.84 μM/5cc.

The decomposition of hydroperoxides to carbonyl is apparently pH-dependent since Schwartz and coworkers have shown that the use of phosphoric acid, on a Celite column to form the DNP's, does not cause the decomposition of methyl linoleate hydroperoxide (5). Table I shows that it is also very much dependent on the temperature at which the reaction is carried out. Similar results were also obtained using cumene hydroperoxide in the presence of hexanal and crotonaldehyde.

These results show that interference can be reduced drastically by using lower temperatures. An added advantage is the fact that ketones give higher derivatization (greater color formation) at the lower temperatures. Table II shows this. Table III indicates that at the lower temperatures crotonaldehyde, hexanal and 2-butanone, representing three types of carbonyl known to occur abundantly in heated fats, can be determined in the presence of one another with greater accuracy.

As modified, the method is identical to that of Henick et al. (1) except for the use of purified tertiary butyl alcohol to dissolve the DNPH reagent in order to obtain lower blanks (6). The reaction is carried out for 20 hours at 5 ± 1C, and the 10.0 ml alcoholic KOH is added with shaking as suggested by Chipault et al. (7). Optical density values

TABLE II

Influence of Temperature on the Molar Extinction of Alkaline Carbonyl 2,4-DNP's

Carbonyl	Molar extinction (E): 30 Min, 60C: 430 mμ	460 mμ	2 Hr, 23C: 430 mμ	460 mμ	20 Hr, 5C: 430 mμ	460 mμ	Literature values: 430 mμ	460 mμ
Hexanal	18,700	14,400	18,800	14,600	19,500	14,950	20,930 [a]	15,290
Crotonaldehyde	25,400	28,050	22,000	28,000	21,950	28,250	23,670 [a]	30,670
Acetone	11,600	8,700	12,300	9,400	20,400	15,700	19,000 [b]	.....
2-Butanone	5,770	4,500	6,760	5,250	18,950	13,600		
Levulinic acid	3,850	2,600	4,650	3,100	12,460	8,900	17,000 [b]	.....
2,3 Pentanedione [c]	9,600	13,750	10,300	13,800	10,620	17,600		

[a] See reference 8.
[b] Reference 9.
[c] Maximum wave-length = 505; E = 17,200; 18,400 and 23,600 at 60, 23 and 5C, respectively (mono-derivative E500 = 23,500 [b]).

TABLE III

Optical Density of Mixed Carbonyl 2,4-DNP's

Conditions	Sample code	Optical density					
		430 mμ			460 mμ		
		Calculated	Found	% Error	Calculated	Found	% Error
30 min, 60C	C-H	1.121	1.105	−1.40	1.174	1.153	−1.79
	C-B	0.754	0.795	+5.45	0.894	0.923	+3.24
	B-H	0.689	0.750	+7.41	0.540	0.578	+7.03
	C-B-H	1.282	1.305	+1.80	1.304	1.328	+1.84
	Average	.....	.....	±4.02	.....	.....	±3.48
2 hr, 23C	C-H	1.121	1.115	−0.49	1.174	1.156	−1.53
	C-B	0.780	0.790	+1.28	0.909	0.923	+1.54
	B-H	0.715	0.735	+2.80	0.555	0.558	+0.54
	C-B-H	1.308	1.240	−5.20	1.319	1.323	+0.30
	Average	.....	.....	±2.44	.....	.....	±0.98
20 hr, 5C	C-H	1.121	1.124	+0.03	1.174	1.155	−1.62
	C-B	1.118	1.080	−3.22	1.142	1.140	−0.18
	B-H	1.053	1.030	−2.00	0.788	0.797	+1.14
	C-B-H	1.646	1.585	−3.10	1.552	1.525	−1.74
	Average	.....	.....	±2.09	.....	.....	±1.17

C = Crotonaldehyde
H = Hexanal
B = 2-Butanone

for acetone, crotonaldehyde and hexanal fit the latter's equations quite well and these have been used to compute the amounts of saturated and unsaturated carbonyl in heated oils.

JOSEPH A. FIORITI
Technical Center
General Foods Corporation
White Plains, New York 10603

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Gas Chromatographic Determination of Chain-Length Distribution in Fatty Acid Ethanolamides

A rapid procedure patterned after the transesterification method described by Peisker (1) for direct preparation of methyl esters from triglycerides was applied to fatty acid ethanolamides. The method is useful in studies of the relationship of chain length distribution to detergent performance.

Approximately 15 mg of fatty acid ethanolamide was weighed into a 4 in. x 7/16 in. O.D. calibrated glass stoppered test tube (Excelo), and 3 ml of methylating reagent added. The test tube was then placed in a pressure tube fabricated from ½ in. O.D. copper tubing and standard plumbing joints and the seal screwed up to finger tightness. The tube was then placed in a heating block (8 in. x 3½ in. aluminum billet drilled to accept the pressure tubes and heated electrically) for 15 minutes at 185C. Pressure tubes were removed, cooled under running water, and the glass tube removed. The contents of the tube were concentrated to 1.5 ml by immersion in a water bath and 1 ml portions of distilled water and 40–60C petroleum ether were added. The contents of the tube were shaken and the petroleum ether layer was transferred to a 7.5 x 0.8 cm round bottom sample tube with the aid of a dropper and the residue reextracted with 1 ml of petroleum ether. The two extracts were combined and methyl esters obtained by evaporation of the solvent.

Gas chromatographic separations were carried out at 170C on a "Pye" Argon chromatograph using a 4 ft 100/120 mesh Celite column containing 10% (w/w) polyethyleneglycoladipate. Chain length distribution (relative percent) was determined by cutting and weighing of individual peaks.

Results obtained for commercial samples of coco mono- and diethanolamides are shown in Table I. The results obtained by direct conversion were in good agreement with those from methylation of the isolated fatty acids. The direct conversion method is more rapid since methyl ester formation requires only 15 minutes. Careful control of the sulfuric acid content of the methylating reagent is essential for retention of liberated free amines in the aqueous phase.

TABLE I

Chain Length Distribution of Fatty Acid Ethanolamides

Carbon No.	CMEA[1]		CDEA[2]	
	a	b	a	b
8	0.97	0.67	4.62	4.30
10	4.04	4.09	6.75	6.64
12	54.97	55.28	56.83	57.13
14	18.91	19.15	16.89	16.93
16	8.87	8.60	6.99	7.18
18	12.24	12.21	7.92	7.82

[1] Coco monoethanolamides.
[2] Coco diethanolamides.
[a] By direct conversion.
[b] By methylation of isolated acids.

S. LEE
N. A. PUTTNAM
Research and Development Dept.
Colgate-Palmolive Ltd.,
Manchester 5, England

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