Stability of lipids in lyophilized rat livers*

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» Oxidation and hydrolysis are probably the main mechanisms of deterioration in lipids of stored tissues. Enzymatic hydrolysis of phospholipid has been observed even in frozen cod flesh stored at −29° (1). We have developed and tested a method for lyophilization and storage of rat liver that preserves the lipids in the condition found in fresh liver extract. This procedure is rapid and convenient, and very little change in lipid composition occurs for at least four months. We found that gross analyses (total lipid, total cholesterol, or total lipid phosphorus) revealed little about important changes in composition that may occur; therefore, fractionation and fatty acid analyses were used to test the storage and preservation procedures.

Fatty livers containing highly unsaturated fatty acids were considered to furnish a most rigorous test of the method. Livers were taken from three castrated male Long-Evans rats, which, for three weeks, had been fed a diet containing 10% safflower oil and 1% cholesterol. A weighed portion of each liver was homogenized immediately with 50–100 ml 95% ethanol, and extracted three times for 1 hr with 95% ethanol. The tissue residue was extracted with diethyl ether for 12 hr in a Soxhlet apparatus to obtain a small but significant amount of lipid having a different fatty acid composition. The extracts were combined and evaporated to dryness in a vacuum evaporator, and the dried lipid was

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TABLE 1. GROSS LIPID COMPOSITION OF FRESH AND LYOPHILIZED RAT LIVERS

Rat No.	Treatment of Liver	Wet Wt*	Per Cent of Wet Weight			
			Total Lipid	Total Cholesterol	Free Cholesterol	Phospholipid†
		g				
125	Extracted fresh	4.371	16.1	4.42	0.37	3.55
"	0°, vacuum 4 mo	2.224	16.9	4.49	0.34	3.26
"	0°, vacuum 9 mo	1.525	16.9	4.30	0.33	2.70
128	Extracted fresh	4.106	12.1	3.47	0.31	3.90
"	0°, N_2 4 mo	1.543	12.2	3.61	0.29	3.89
"	0°, N_2 9 mo	0.906	13.2	3.66	0.31	3.59
141	Extracted fresh	4.133	11.4	1.75	0.27	3.84
"	RT, vacuum 4 mo‡	1.694	10.0	1.61	0.23	3.84
"	RT, vacuum 9 mo	1.742	11.0	1.72	0.22	3.23

* The wet weight of the lyophilized samples was calculated from the average weight loss after lyophilization, which was 63.3% of wet weight.

† Phospholipid = lipid phosphorus × 25.

‡ RT = room temperature.

reextracted into petroleum ether for storage at 0° or below until analysis. The rest of each fresh liver was immediately frozen with solid carbon dioxide. The frozen livers were lyophilized for 24 hr in a Napco[1] vacuum oven (Model 58301) that had been modified so that a 1-in. i.d. tube connected it to a trap surrounded with solid carbon dioxide in isopropyl alcohol. The system was evacuated to 0.5–0.3 mm Hg by an oil vacuum pump (Welch Duoseal).[2] The liver samples were stored intact under three conditions: (1) in an evacuated desiccator at 0°, (2) in a desiccator flushed with N_2 at 0°, or (3) in an evacuated desiccator at room temperature. Portions were removed after four and nine months, powdered with a mortar and pestle, and extracted in the same manner as the fresh liver samples. No water was added to reconstitute the dry tissue.

These petroleum ether extracts, and those prepared earlier and stored, were analyzed for total lipid (2), free and total cholesterol (3), and lipid phosphorus (4). The results of these analyses are shown in Table 1. Lyophilization and storage had no apparent effect on the gross composition except for a decrease in lipid phosphorus after nine months of storage.

The lipid extracts were fractionated on silicic acid (5, 6) into cholesterol esters; triglycerides; phospholipids; and a mixed fraction containing mono- and diglycerides, free fatty acids, and free cholesterol. Fatty acids were determined by gas-liquid chromatography[3] (GLC) on all fractions (7); and total lipids, cholesterol (8), and phosphorus were measured on appropriate fractions. The gross determinations and the values based on recovery from fractionation agreed within experimental error in all cases, showing that lyophilization and storage did not affect the gross fractionation of the lipids.

Since oxidative degradation of the unsaturated fatty acids was expected to be the most damaging effect of storage, the fatty acid composition of each fraction was investigated. Four months of storage had no consistent effect on the fatty acid compositions of cholesterol esters, triglycerides, or phospholipids — not even on the highly unsaturated components that might be expected to deteriorate most rapidly. In the small mixed fraction, however, the proportion of linoleic acid increased. This minor fraction comprised less than 10% of the total lipid, so that a partial conversion of the large triglyceride fraction to mono- and diglycerides and free fatty acids, for example, could have altered the composition of the mixed fraction without noticeably affecting the triglyceride fatty acid pattern or lipid recovery value.

After nine months of storage in the lyophilized state, definite changes appeared in the fatty acid patterns of all fractions. The proportion of arachidonic acid decreased from an average fresh value of 45 to 20% of the fatty acids in the phospholipid fraction, and from 7.3 to 0.9% in the cholesterol ester fraction. In the cholesterol ester, the proportion of linoleic acid decreased from a fresh value of 52 to 24%. The fatty acid compositions of the triglyceride and mixed fractions changed considerably less than those of the phospholipid and cholesterol ester fractions. An increased proportion of saturated components in the latter fractions compensated for the disappearance of the unsaturated fatty acids.

[1] National Appliance Company, Portland, Oregon. Detailed descriptions given in chemical equipment catalogues.

[2] This apparatus can lyophilize 50 rat livers in 24 hr.

[3] Aerograph Model A-90-C, Wilkens Instrument and Research, Inc., Walnut Creek, California. The liquid phase was poly-(diethylene glycol succinate) on Chromosorb W.

The various conditions under which the samples were stored seemed to have no effect on the rate of lipid degradation. Therefore, it appears that lyophilized livers can be stored in a desiccator without air for at least four months with little or no destruction of the lipids. Results obtained in these experiments are probably applicable to other tissues, but the procedure should be tested on each tissue. In our experience, as well as that of others (9), procedures that are suitable for the quantitative extraction of liver lipids do not necessarily give equally good results with plasma from the same species.

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