Effect of Storage on Lipids of Fish Protein Concentrate

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ABSTRACT

Fish protein concentrates (FPC) made from pout, alewife and Gulf menhaden were stored for 6 months at temperatures of 37 C and 50 C with no attempt to control humidity. When the amount of extractable lipid was 0.1% or less there were small changes in the lipid pattern: a small decrease in the amount of neutral lipid-free fatty acid fraction and larger decreases in the long chain, unsaturated fatty acids (C20:5, C22:6 for pout and C20:5 for alewife). In the FPC sample containing residual lipid of about 0.5% there were decreases in the amount of lipid extractable after 6 months, appreciable in the 37 C sample, and greater in the sample stored at 50 C. These samples also showed decreases in the neutral lipid plus free fatty acid fractions and decreases in the long chain, unsaturated fatty acids C20:5 and C22:6.

INTRODUCTION

The nature of the residual lipids in fish protein concentrates (FPC) made from red hake *(Urophycis chuss)* and Atlantic menhaden *(Brevoortia tyrannus)* by isopropanol extraction have been described previously (1,2). Here we discuss the effects of storage for 6 months at 37 C and 50 C on the extractable lipids of FPC made from pout *(Macrozaorces americanus)*, alewife *(Alosa pseudoharengus)*, and Gulf menhaden *(Brevoortia patronus)*.

METHODS

The products were prepared and supplied by College Park Fishery Products Technology Laboratory, National Marine Fisheries Service. Triplicate samples (200 g each) of pout and alewife FPCs containing approximately 0.1% lipid and of Gulf menhaden FPC containing approximately 0.5% lipid were placed in one liter beakers, covered with watch glasses and stored in constant temperature ovens at 37 C and 50 C for 6 months, then held at room temperature until extraction. During these procedures humidity was not controlled. These and control samples were analyzed in the following manner: Samples (200 g) were extracted in duplicate or triplicate in large Soxhlet extractors with chloroform-methanol (2:1 v/v) for a total of two weeks (1). The extracts were kept in an oxygen free atmosphere by bubbling nitrogen through a side arm tube into the extraction flask. Solvent was removed in vacuo, the residue was dissolved in chloroform-methanol (19:1) saturated with water, and run through a Sephadex G-25 column for removal of nonlipid contaminants (3) with a glass-Teflon column (Chromatronix Model LC-1) and the injection and controlled pump system described by Van der Veen et al. (4).

Aliquots of total lipid (70-100 mg) were chromatographed on activated (2 hr at 110 C) Mallinckrodt SilicAR CC-7, 100-200 mesh, columns to separate fractions containing mainly (1) neutral lipids and free fatty acids, (2) sphingolipids and (3) phospholipids. One- and two-dimensional thin layer chromatography (TLC) was used to identify the lipid components of the column fractions (1).

Twenty to 50 mg of total lipid was saponified (5) and methylated for characterization of fatty acids by gas chromatography (1). A Hewlett-Packard Model 810 gas chromatograph with a 6 ft x 1/4 in. i.d. silanized Pyrex column packed with 10% diethylene glycol adipate on Gas Chrom Q, 80/100 mesh (Applied Science Laboratories) was used for these analyses.

RESULTS

After 6 months storage the amount and character of the extracted residual lipid had not changed appreciably in the FPC samples having low total lipid (Table I). Pout FPC yielded 0.11% lipid both before and after storage for 6 months at 50 C. The extract of the pout samples that had

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TABLE I

Total Lipid and Silicic Acid Column Chromatography[a]

Sample	Storage conditions	Total lipid, %	Column fractions, % total lipid		
			Fraction 1	Fraction 2	Fraction 3
Pout[b]	Control	0.106	67.9	13.7	18.3
	50 C, 6 mo	0.105	64.0	18.0	18.0
Alewife	Control	0.065	62.9	27.4	9.7
	37 C, 6 mo	0.069	50.8	37.1	12.0
	50 C, 6 mo	0.073	56.0	31.1	13.0
Gulf menhaden	Control	0.56	86.3	10.0	3.8
	37 C, 6 mo	0.50	82.3	11.9	5.9
	50 C, 6 mo	0.46	81.9	12.7	5.6

[a]Figures are averages of three separate values: fraction 1 (eluted with chloroform and chloroform plus 2% methanol) was mainly triglyceride, fraction 2 (eluted with chloroform plus 18% methanol) was mainly sphingolipid and fraction 3 (eluted with chloroform plus 25% methanol and chloroform plus 50% methanol) was mainly phospholipid (cf. [1]).

[b]The 37 C, 6 mo extract was contaminated (see text).

TABLE II

Effect of Storage on Apparent Fatty Acid Composition of Fish Protein Concentrates Total Lipids[a]

Fatty acid[b]	Pout			Alewife			Gulf Menhaden		
	Control	6 mo, 37 C	6 mo, 50 C	Control	6 mo, 37 C	6 mo, 50 C	Control	6 mo, 37 C	6 mo, 50 C
14:0	2.5	3.4	3.2	6.6	7.4	7.4	10.7	12.4	12.8
16:0	16.8	18.6	19.8	23.1	23.6	24.1	26.4	28.8	29.7
16:1	11.2	11.2	11.6	13.7	13.5	13.6	14.8	15.2	15.4
18:0	4.7	5.6	5.9	4.1	4.0	4.0	5.8	5.6	5.5
18:1	26.6	27.6	29.0	25.7	25.6	26.2	18.2	18.8	18.7
18:2	0.5	0.6	---	2.9	2.6	2.5	0.7	0.5	0.5
20:1	2.2	2.3	2.2	2.0	1.8	1.8	2.6	2.5	2.6
20:4	1.8	1.5	1.4	2.2	2.1	2.0	0.4	0.3	0.3
20:5	17.8	13.1	12.3	5.6	4.7	4.7	8.4	6.2	5.7
22:6	8.8	6.8	6.3	4.2	4.1	4.1	3.5	2.6	2.3

[a]As per cent of total fatty acids.

[b]Number of carbon atoms: number of double bonds. Additional fatty acids, tentatively identified but present in amounts of 2% or less, were: 12:0, 15:0, anteiso 15:0, iso 16:0, 16:2, 17:0, iso 18:0, 18:3, 18:4, 19:0, iso 20:0, 20:2, 21:0, 22:1, 22:2, 22:3, 22:4, 22:5, 24:1.

been stored at 37 C was contaminated during the extraction process by components of latex tubing, verified by two-dimensional TLC. Fractions obtained by silicic acid column chromatogrpahy of the total lipid extract showed a slight decrease after storage from the control in neutral lipids and a concomitant increase in the sphingolipid fraction.

The average extractable lipid in alewife FPC also was not changed during storage (Table I). Results of column chromatography showed an apparent decrease in the per cent of neutral lipid (fraction 1) with relative increase of sphingolipid (fraction 2) and phospholipid (fraction 3).

The unstored Gulf menhaden FPC contained 0.56% total lipid; the stored samples at 37 C and 50 C contained 0.50% and 0.46% respectively. The decrease in lipid perhaps reflected the formation of lipid-protein complexes or some breakdown of lipid into smaller fragments that were either volatile or lost during Sephadex chromatography. Column chromatography of Gulf menhaden FPC lipid, as for pout and alewife. showed an apparent decrease in fraction 1 and an increase in fractions 2 and 3.

The trend of generally decreasing values of fraction 1 during storage suggests that the simple lipids are less stable than the complex lipids. The apparent phospholipid increase was 25% for alewife and 32% for menhaden. Of the three samples, alewife FPC appeared to be the most stable by this storage test.

Gas chromatography of the total fatty acids in the samples before and after storage showed an apparent increase in the lower molecular weight fatty acids with the major disappearance occurring in C20:5 and C22:6 (Table II). In pout, C20:5 dropped by 26% when stored at 37 C and by 31% at 50 C; in alewife it decreased by 16% in both storage groups; and in Gulf menhaden it showed a 26% lower value at 37 C and 32% at 50 C. Similar changes were found for C22:6 in pout and Gulf menhaden but C22:6 appeared unchanged in alewife FPC, another example of its greater stability.

This study shows that lipids in FPC are relatively stable when they are present at levels of 0.1% or less. With an FPC containing 0.5% lipid the changes during storage at 37 C and 50 C were reflected in measurable losses in extractibility and more severe losses in content of the highly unsaturated fatty acids.

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