Technical

Preparation of an ω3 Fatty Acid Concentrate from Cod Liver Oil

N. HAAGSMA[1], C.M. van GENT[2], J.B. LUTEN[3], R.W. de JONG[1] and E. van DOORN[2]

ABSTRACT

Preparation of an 85% ω3 fatty acid concentrate from cod liver oil is described. The urea inclusion compound method was used. On the average, 265 g of concentrate was obtained from 1,000 g of cod liver oil. For stabilization of the fatty acids, 0.01% octyl gallate was added.

INTRODUCTION

In a recent study on the dose-response relationship between ω3 fatty acid intake and certain blood parameters in human subjects, an ω3 fatty acid concentrate was used (1,2). Such a concentrate was preferred to "whole" fish oil because it keeps the daily amount of fatty acids ingested as low as possible and other fatty acids, which might be physiologically active, are then present only in small amounts or are completely absent.

From among the different methods available for the preparation of concentrates of polyunsaturated fatty acids from natural sources (3), low-temperature crystallization and urea inclusion compound formation (4-7) are suitable, as they allow the handling of large quantities of fatty acids (ca. 3,000 g of ω3 fatty acids were needed [1,2]).

In preliminary experiments, the method of urea inclusion compound formation was found to give better results with respect to yield and fatty acid composition in the concentrate. The urea fractionation of the fatty acids is mainly based on the degree of unsaturation: the more unsaturated, the less they will be included in the urea crystals.

MATERIALS AND METHODS

Saponification

One thousand g of cod liver oil (purchased from a local firm) was mixed with 2 L of a NaOH solution in water/ethanol and heated, with stirring, for 30 min at 60 C. The NaOH solution was prepared by dissolving 480 g of sodium hydroxide and 5 g of Na_2EDTA in 1.6 L of water. To this solution, 1.6 L of ethanol was added. After saponification, 400 mL of water was added.

Extraction of Fatty Acids

Hexane (4 L) was added and, after stirring for 1 hr, the upper layer (ca. 1.6 L) containing unsaponifiable matter was removed and discarded. Concentrated hydrochloric acid was added to the lower layer, with stirring, until it had pH 1. Again, 2 layers appeared. The lower phase was discarded and the upper hexane layer was evaporated to a small volume in a vacuum rotary evaporator at 30 C.

[1]Department of the Science of Food of Animal Origin, Section Chemistry, Faculty of Veterinary Medicine, The University of Utrecht, The Netherlands.
[2]Gaubius Institute, Health Research Organization TNO, Leiden, The Netherlands.
[3]Institute for Fishery Products TNO, Ymuiden, The Netherlands.

Formation of Urea Inclusion Compounds

The fatty acids were added under constant stirring to a hot (60-65 C) solution of 3,000 g of urea in 8 L of methanol. If necessary, the solution was heated until clear. Urea and urea complexes were allowed to crystallize overnight at room temperature. The solution was filtered, and the filtrate was kept at 4 C for 3 hr and then filtered again.

Extraction of ω3 Fatty Acids

To each 3 L of filtrate, 1.0 L of hexane and 0.5 L of concentrated hydrochloric acid were added and the mixture was thoroughly stirred for 1 hr. The hexane layer was separated. About 1.5 L of water was added to the lower layer. This layer was extracted again with 1 L of hexane. The extracts were combined.

Stabilization of ω3 Fatty Acids

Octyl gallate (0.01% on a fatty acid base) was added to increase the stability of the fatty acids and the extracts were stored under nitrogen at -25 C. Shortly before use, the hexane was removed by evaporation under vacuum at 30 C. The remaining concentrate was packed in gelatin capsules and stored at 4 C. Each capsule contained ca. 0.5 g of fatty acid concentrate. The fatty acid composition was determined by GLC according to IUPAC II.D.19 and II.D.25 and the peroxide value according to IUPAC II.D.13.

RESULTS AND DISCUSSION

The fatty acid composition of cod liver oil and the mean composition of batches of ω3 fatty acid concentrate are given in Table I. About 85% of the fatty acids in the concentrate was ω3 fatty acids. It is remarkable that the concentration factor of 20:5ω3 is smaller compared to that of 18:4ω3 and 22:6ω3. This was found in all the prepared concentrates. This phenomenon cannot be explained.

On the average, 265 g of concentrate was obtained from 1,000 g of cod liver oil. Thus, the procedure has a mean overall efficiency of 82% as related to the ω3 fatty acids present in the starting material.

Saponification had to be performed in as small a volume as possible during the large-scale procedure, resulting in a solution with maximal content of sodium hydroxide. Moreover, an optimal proportion of cod liver oil to saponification solution was used, allowing the reaction in a one-phase system. EDTA was used to bind traces of copper and iron, as these catalyze autoxidation. A relatively large volume of hexane had to be added to the solution of soaps to obtain a phase separation, thus removing unsaponifiable matter (sterols, chlorinated hydrocarbons). After acidification of the lower phase, the remaining hexane separated, extracting the fatty acids nearly quantitatively. A second extraction with additional hexane yielded only a small amount of fatty acids.

TABLE I

Fatty Acid Composition (%) of Cod Liver Oil and Mean Composition of Batches of ω3 Fatty Acid Concentrate

	Cod liver oil	Concentrate
14:0[a]	4.7	1.0
16:0	11.4	–
16:1	9.1	1.3
16:2	0.9	2.0
18:0	2.2	–
18:1	24.9	2.0
18:2	1.8	1.6
18:3ω3	1.0	1.0
18:4ω3	2.4	10.0
20:1	12.0	–
20:3ω3	0.1	0.7
20:4ω3	–	1.6
20:5ω3	12.1	27.6
22:1	4.8	–
22:4	–	2.8
22:5ω3	–	1.2
22:6ω3	11.7	44.6
Σ	99.1	97.4
Σ ω3	27.3	86.7

[a]No. carbons:no. double bonds.

The formation of urea inclusion compounds was performed in a nearly saturated solution of urea in methanol at 60-65 C. We have studied the influence of the urea/fatty acid (w/w) ratio on the percentage of ω3 fatty acids in the concentrate. Maximal efficiency (82%) was found when the ratio was about 3. This means that rather large volumes had to be handled. The yield of the extraction of the ω3 fatty acids from the methanolic solution was enhanced by a second extraction with hexane as described in Materials and Methods. This resulted in an increase of ca. 20% in the efficiency of the procedure. Various antioxidants to stabilize the ω3 fatty acids were investigated. Octyl gallate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ), were tested with the Schaal test (8) at 25 C. Octyl gallate and TBHQ were found to be superior to BHA and much superior to BHT. Octyl gallate was chosen, as the use of TBHQ in fats for human consumption is not allowed in the Netherlands.

The ω3 fatty acids should be kept in the hexane solution at -25 C as long as possible, as it has been found that the peroxide value under these conditions hardly increases. The peroxide value of the concentrate usually amounts to 4-6. Storage of the encapsulated concentrate for 2 weeks at 4 C resulted in only a small increase in peroxide value.

Polymers in the concentrate were not detectable by gel permeation chromatography (GPC). This means that the content was less than .5%. However, 2 peaks, one corresponding to the methyl esters of the fatty acids and the other to the fatty acids, were found in the GPC analysis. It was estimated that one-third of the fatty acids was present as methyl esters. The presence of methyl esters was confirmed by gas liquid and thin layer chromatographies. These methyl esters originated during the urea inclusion complex formation step. In our dose-response study (1,2), there was no objection to this methylation. It can possibly be avoided by substituting methanol with another solvent, e.g., ethanol, during the urea inclusion compound formation.

REFERENCES

1. van Gent, C.M., J.B. Luten, H.C. Bronsgeest-Schoute and A. Ruiter, Lancet 8154:1249 (1979); 8159:108 (1980).
2. Bronsgeest-Schoute, H.C., C.M. van Gent, J.B. Luten and A. Ruiter, Am. J. Clin. Nutr. 34:1352 (1981).
3. Privett, O.S., in "Progress in the Chemistry of Fats and Other Lipids," Vol. IX, part 3, edited by R.T. Holman, Pergamon Press, 1968, p. 407.
4. Bauchart, D., and B. Aurousseau, JAOCS 57:121 (1980).
5. Aurousseau, B., and D. Bauchart, Ibid. 57:125 (1980).
6. Abu-Nasr, A.M., W.M. Potts and R.T. Holman, Ibid. 31:16 (1954).
7. Domart, Cl., D.T. Miyauchi and W.N. Sumerwell, Ibid. 32:481 (1955).
8. Pardun, H., and E. Kroll, Deutsche Lebensm. Rundsch. 66:413 (1970).

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