Enrichment of Gamma Linolenic Acid from the Fungal Lipids

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Abstract- *Mortierella ramanniana* is known as a gamma linolenic acid as an gamma linolenic acid producing oleaginous fungus. Enrichment of GLA from the fungal lipids by a urea inclusion method was studied. Most of the saturated and monosaturated, 93.0% and 84.6%, respectively were removed by forming urea inclusion compounds. GLA was concentrated after urea inclusion. Its content in total fatty acids increased 6.2 folds and recovery of reached 57.1% with a recovery of 90%.

Index Terms- Gamma Linolenic acid; Enrichment; Fungal lipids; *Mortierella ramanniana*; urea inclusion

I. Introduction

Omega 6 long chain fatty acids have in recent years, become increasingly popular in the nutritional supplement and nutraceutical arena, with the release of an increasing number of new nutritional supplements and functional foods. Over the past decade, consumers have begun to recognize the importance of these highly unsaturated fatty acids within their diets. Of the omega 6 oils, Gamma Linolenic acid (GLA; C18: 3n-6) and arachidonic acid (ARA; C20: 4n-6) constitute the largest number of beneficial health claims.[1]

Currently, the major source of these fatty acids is the consumption of plant oils such as evening primrose, black currant, Borage. These oils, accumulated by plants, are ultimately derived from microorganisms. One area receiving considerable interest at present is the cultivation of polyunsaturated fatty acids (PUFA)-producing fungi. The filamentous fungus *Mortierella ramanniana* belonging to the phycomycetes class has been identified as a promising producer of GLA.[2-3]

This study dealt with extraction of lipids from *M.ramanniana* and enrichment of GLA from fungal lipids by a urea inclusion method.

II. Material and Methods

Mycelium biomass

The *M.ramanniana* was grown in ten 1 L Erlenmeyer flasks. The medium consisted of (g/l) glucose 100; peptone 10 and yeast extract 1.The pH of the medium was adjusted to 6.5 before autoclaving. The fungal cells were harvested by suction filtration, washed with water thrice. Then the wet biomass was used for direct extraction of lipids.

Extraction of lipids

Extraction of lipids from the biomass was performed according to the procedure of Blight and Dyer (1959)[4] using chloroform/methanol (2:1). Chloroform phase was recovered .The same process was repeated thrice. The whole solvent was evaporated and dried under vacuum.

Oil saponification and preparation of fatty acids

Fungal oil (10g) was saponified with stirring in 100ml 95% ethanol, 5g NaOH, 100ml water at 75°C for 1 h. 1N HCl was then added to set pH in the range of 2.0-2.5.The lower layer was removed and discarded. The upper layer was fatty acid.

Enrichment of GLA from fatty mixture by urea inclusion

Fatty acids (5g) were added to a hot (75°C) solution of 20g urea in 50ml ethanol. The solution of 20 g urea in 50 ml ethanol. The solution was heated and stirred until clear urea and urea compounds were allowed to crystallize at room temperature overnight. The precipitate was removed with filtration and washed with urea-saturated ethanol. The filtrate was then mixed with 40 ml hexane and 40 ml 0.1 N HCl, and the hexane layer was separated and evaporated to obtain GLA concentrate.

Assay

Fatty acids were methylated according to Christopher son and Glass [5] method and fatty acid methyl esters were determined by using gas chromatograph Agilent 6890 Series Gas Chromatograph equipped with a FID and the capillary column DB-23 (30 m X 0.25 mm i.d. X 0.5 μm film thickness; J & W Scientific, USA). The injector and detector temperatures were maintained at 230 and 250°C respectively. The oven was programmed for 2 min at 160°C to 180 °C at 6 °C/min, maintained for 2 min at 180 °C, increased further to 230 °C at 4 °C/min and finally maintained for 10 min at 230 °C. The carrier gas, Nitrogen was used at a flow rate of 1.5 mL/min. The injection volume was 1 μL, with a split ratio of 50:1.Identification of fatty acid methyl esters were made using GC-MS was performed with Agilent 6890N Gas Chromatograph connected to Agilent 5973 Mass Spectrometer at 70 eV (m/z 50-550; source at 230°C and quadruple at 150°C) in the EI mode with a HP-5ms capillary column (30 m X 0.25 mm i.d. X 0.25 μM film thickness; J & W Scientific, USA). The carrier gas, Helium was used at a flow rate of 1.0 mL/min. The inlet temp was maintained at 300°C and the oven was programmed for 2 min at 150°C to 300°C at 4 °C/min, and maintained for 20 min at 300°C. The injection volume was 1 μL, with a split ratio of 50:1. Structural assignments were based on interpretation of mass spectrometric fragmentation and confirmed by comparison of retention times as well as fragmentation pattern of authentic compounds and the spectral data obtained from the Wiley and NIST libraries.

III. Results and Discussion

Enrichment of GLA by urea inclusion

The lipids in the fungal cells were obtained by wet extraction using the Bligh and Dyer (1959) method, the solvents were chloroform/methanol and lipids yield was 35.725%.

Urea inclusion is an enrichment technique for polyunsaturated fatty acids mainly based on the degree of unsaturation of the fatty acids. The reported optimal experimental conditions (4:1 urea/fatty acid ratio and 28°C crystallization temperature) for enrichment of AA from micro algae (Gimenez et al., 1998) [6] were applied in this study. The results of enrichment of GLA were shown in the table1. Saturated and monounsaturated fatty acids easily formed urea compounds to be removed entirely (93.0%); most of the monounsaturated fatty acids (about 84.6%) was also removed. Unsaturated fatty acids formed their double bonds and so remained in the concentrate. Polyunsaturated fatty acids in urea concentrate increased significantly. The GLA content increased 6.2 folds and reached 57.1% with a recovery of 90%. Therefore, the urea inclusion method is an appropriate way for GLA enrichment from fungal lipids, considering both concentration factor and recovery. Since it is difficult to separate GLA from other polyunsaturated fatty acids by urea inclusion, more effective purification techniques, e.g., chromatography should be applied to obtain high purity GLA.

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Table 1
Fatty acid composition of urea concentrates from fungal lipids of *Mortierella ramanniana*

Fatty Acid	Fungal Lipids	Urea Concentrate
14:0	1.3	-
16:0	36.8	0.3
18:0	2.5	0.9
18:1	41.5	7.6
18:2	5.2	4.3
18:3	9.1	57.1
20:4	3.9	7.4
Total saturates		
Recovery of GLA(%)		