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Urea Fractionation Method for Concentration of Fatty Acid Extract of *Spirulina Platensis*

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A cheap and reliable method for concentration of unsaturated fatty acid (γ-linolenic acid) from *Spirulina platensis* has been demonstrated using urea and methanol. Using this method, γ-linolenic acid purity was enhanced from 24-62% of the total fatty acids.

Keywords: Urea, methanol, γ-linolenic acid, *Spirulina platensis*.

γ- linolenic acid is an important metabolite, which finds wide application in treating various disorders: rheumatoid arthritis(Soeken 2004), eczema(Wright and Bolton 1989), diabetes, multiple trauma, and premenstrual syndrome (Wantanabe *et al*., 2005). The methyl ester extract from *Spirulina platensis* can be concentrated using urea adduct method (Mahajan and Kamat, 1995). As an application, this procedure was useful to prepare a lipid extract enriched in γ-linolenic acid from seed oil of Boraginaceae species (Campra-Madrid P *et al.,* Chromatographia 2002, 56, 673). A simple procedure used to enrich fish oil in EPA and DHA was also reported for squid visceral oil ethyl esters (Hwang LS *et al.,* JAOCS 2001, 78, 473).

When complex fatty acid mixtures are analyzed or when information on the unsaturation degree is needed, a simple fractionation of the crude fatty extract can be achieved by urea adduct formation. Upon crystallization, urea forms inclusion complexes with some long-chain aliphatic compounds. Saturated fatty acids as fatty acid methyl esters form complexes readily (as trans fatty acids), their formation being less efficient with increasing number of double bonds or in the presence of branched chains. This procedure cannot be used as an analytical technique but is frequently applied to obtain a concentrate of polyunsaturated or branched-chain fatty acids. This work demonstrates the urea fractionation as a possible method for concentration of essential fatty acids from *Spirulina platensis.*

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EXPERIMENTAL

Fatty acid extraction and methylation

Fatty acids were extracted using single step direct trans-methylation of freeze-dried algae using the method of Cohen *et al* (1993). Heptadecanoic acid was used as internal standard.

Fatty acid analysis and Quantification

The fatty acid composition (Fig. 1) was analyzed using Gas chromatography (GC make-Chemito HR 5820). The fatty acids were identified by correlating the retention time with that of standard fatty acids (Sigma &Co). The column used to identify fatty acids was an EGSS packed column with 10 % w/w chromosorb solid support (Mahajan and Kamat 1995). The column was operated at 180^{o}C in an isothermal mode. The mobile phase was an inert nitrogen gas fixed at a

flow rate of 35 ml per min. Injector and detector temperatures were kept at 250°C. The detector of gas chromatography was flame ionizing detector. Fatty acid quantification was done using internal standard method.

Urea fractionation method

About 100mg of methylated fatty acid ester was dissolved in 10 ml of methanol containing 4g urea. In addition, methanol /urea ratio was maintained in the ratio of 3:1(v/w).

The fatty acid urea mixture was subjected to heating until clear. The urea and urea inclusion complex were allowed to crystallize at room temperature for 5-6 hours, excess methanol was added to the vial containing urea inclusion complex. The resulting mother liquor was filtered using whatman#1 filter paper. To the filtrate, hexane was added and the unsaturated fatty acids were extracted by two washings of hexane (5 ml). Fatty acids recovered from the hexane phase and quantitatively estimated by using Gas Chromatography.

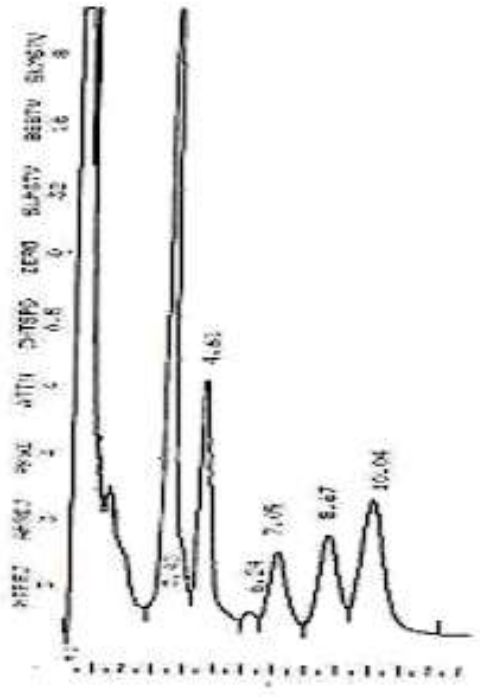

Fig. 1. Fatty acid profile of *Spirulina platensis* ARM740 showing various fatty acids and their retention times. Palmitic acid (3.43); Heptade-conoic acid(internal standard) (4.61) Stearic acid (6.24); Oleic acid (7.09); Linoleic acid (8.67); GLA (10.04)

RESULTS AND DISCUSSION

In the present study *Spirulina platensis* ARM 740 and a commercial sample (Sunnova) were considered as sources of methyl esters Exploiting the ionic interaction of urea and crystal formation with saturated fatty acids, unsaturated fatty acids were separated as an unbound form from the dissolved solvent (methanol).The fatty acid concentration by urea inclusion method was done to obtain GLA in concentrated form. Table 1 shows the fatty acid composition and total fatty acids in both commercial sample (Sunnova) and *Spirulina platensis* ARM740. As can be seen from the data, the essential fatty acid, GLA in commercial sample and in *Spirulina platensis* ARM 740 was 24 % of total fatty acids. Palmitic acid being major fatty acid and oleic acid varying to a greater extent is both samples.

The fatty acid profile obtained before and after treating fatty acid methyl ester with urea-methanol was shown in Table 2. Data shows that urea fractionation removed all the saturated fatty acids except palmitic acid. Trace of palmitic acid was left behind as an impurity along with linoleic and linolenic acids. It was also observed that stearic and oleic acids were completely removed from the methyl ester mixture in both algal samples. The unsaturated fatty acids viz., linoleic acid(C18:2) and linolenic acid (GLA)were left behind as principal fatty acids in the mother liquor, In the first extraction step, the GLA purity in the commercial sample was 54.31% with 58.0% recovery while the fatty acid concentrate from *Spirulina platensis* ARM 740 achieved a purity of 62.7% with a recovery of 62.2%. The reduction in purity of GLA was largely due to the linoleic acid found along with GLA. Table 3 summarizes the purity and yields obtained by Cohen and Cohen(1991), Gimenez Gimenez et al.(1998) and this work. Owing to its low cost, low toxicity, and simplicity, urea fractionation is recommended for large scale concentration of methyl esters of microalgal lipids.

Table 1. Fatty acid composition and its content in various algal sources

Source	Fatty acid composition (%TFA)					Fattyacid content (% dry wt.)	
	16:0	18:0	18:1	18:2	GLA	GLA	TFA
Commercial sample	54.67	0.65	1.7	18.45	24.5	1.17	4.8
Spirulina platensis ARM 740	48.51	0.40	12.3	14.57	24.6	0.86	3.5

Table 2. Urea fractionation method showing purity and recovery of GLA

Sample	Major fatty acids	Area under the peak before under addition	Area under the peak after urea addition	%purityof GLA among fatty acids	% Recovery of GLA	% unsaturation in TFA recorded sample
Commercial sample (Sunnova)	C16:0	6583127	501922	54.31	58.0	81.77
	C18:0	78318	-			
	C18:1	205557	-			
	C18:2	2222281	649329			
	C18:3	2950544	1602619			
Spirulina platensis ARM 740	C16:0	3347443	157372			
	C18:0	334535	-	62.71	62.27	88.46
	C18:1	1315338	-			
	C18:2	1004149	351452			
	C18:3	1374063	855734			

(-) Not detected

Table 3. Essential fatty acids and their purity, yields obtained using urea fractionation

Author	Fatty acids	Organism	Purity	Yield
Cohen and Cohen(1991)	Arachidonic acid	*Porphyridium cruentum*	80.0	-
	Eicosapentaenoic acid	*Porphyridium cruentum*	81.9	-
Gimenez et al(1998)	Arachidonic acid	*Porphyridium cruentum*	34.1	61.8
	Eicosapentaenoic acid	*Porphyridium cruentum*	42.0	67.7
This work	GLA	*Spirulina platensis*	62.7	62.2

Conclusion

As discussed here, in large scale, GLA can be concentrated from the *Spirulina platensis*. The essential fatty acid can be recovered as methyl ester at a purity and recovery state of 62 %.

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