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Polyunsaturated fatty acid enrichment from sardine oil by urea-fractionation and argentation chromatography

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Fish and crustaceans cannot synthesize n3 and n6 long chain polyunsaturated fatty acids (LC-PUFAs) *de novo* from precursor molecules. Of these, eicosapentaenoic acid (EPA), docosahexanoic acid (DHA), and arachidonic acid (AA) are essential in the diet of a majority of marine finfish and crustaceans, especially for the larvae and broodstock. PUFAs are widely available in a large variety of marine organisms like microalgae, polychaetes, finfish and shellfish, but fish oil is easily available, cheap, and contain considerable amount of PUFAs. Crude sardine oil has about 33.26% PUFA and the present study highlights a method for purification of sardine fatty acids with the goal to get a PUFA concentrate for ultimate use in marine fish and crustacean broodstock and larval diets. PUFA concentrates have been prepared from sardine oil by urea fractionation and liquid column chromatography of the urea-concentrate using AgNO3-impregnated neutral alumina as stationary phase. The purity of different fractions and recovery levels were studied in detail using gas liquid chromatography (GLC) and gas chromatography-mass spectrometry (GC-MS).

PUFA, derived from sardine oil (50g) was concentrated by urea-fractionation of saponified free fatty acids using methanol at different temperatures (2, 4 and 6^{0}C) and urea/fatty acid ratios (2:1, 3:1, and 4:1) to recover concentrated PUFAs. The fatty acids were extracted with n-hexane (3 X 100 ml) to cause the phase separation of urea and concentrated PUFAs. Further purification of the concentrated PUFAs was accomplished by argentation neutral alumina column chromatography. The methyl esters of fatty acids were fractionated using 5-50% diethyl ether / n-hexane as eluants. The EPA was purified by passing through the column along with 50% diethyl ether / n-hexane. Free fatty acids were transesterified into their methyl esters by reaction with a methylating mixture before GLC/GC-MS analysis.

Most of the saturated and monounsaturated fatty acids were removed during urea complexation resulting in relatively high level of PUFA (69.22+_ 4.94%). The highest concentration of EPA (purity 47.78%) was obtained using a urea/fatty acid ratio of 4:1 at the crystallization temperature of 4^{0}C, with recovery of > 95%. The purity and yield were relatively

low at crystallization temperatures of 2 and 6^0C, and urea fatty acid ratios of 2:1 and 3:1. Argentation neutral alumina column chromatography resulted in EPA of high purity (99.6%) with an overall recovery of 41.24% using 50% diethyl ether/n-hexane as eluting solvent. The present study thus enabled to develop a process of purification for achieving concentrated PUFA (78.35%) using a urea/fatty acid ratio of 4:1 at the crystallization temperature of 4^0C, with recovery of > 80%.