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Preparation of polyunsaturated fatty acid concentrates from sardine oil by an alkaline Lipase from *Bacillus licheniformis* MTCC 6824

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The n3 and n6 essential polyunsaturated fatty acids (PUFAs) are recognized to have beneficial physiological and nutritional effects. Lipases are biotechnologically valuable enzymes, which specifically hydrolyze carboxyl esters of triglycerides into fatty acids, and are being used as animal feed supplement to increase bioavailability of n3 or n6 polyunsaturated fatty acids. Among microbial lipases, *Bacillus* lipases constitute a major group. A typical example of lipase-mediated modification of fats and

oils is enrichment of PUFAs. Lipases exhibit discriminative ability among different PUFA's either depending upon the number of olefinic double bonds or position of acyl side chain in glyceryl moiety and position of double bonds. The mild conditions and unique substrate specificity of lipase used in enzymatic reactions offer a promising alternative to avoid the oxidation and geometrical isomerization. As fish oils contain mixtures of EPA, DHA, and other unsaturated and saturated fatty acids, there is a great need to

obtain purified PUFA concentrates. The present report highlights a method for enrichment of EPA and AA from sardine oil in one-step hydrolysis by *Bacillus licheniformis* MTCC 6824 lipase, and further concentrating the fatty acids by urea fractionation.

The lipase produced by *Bacillus licheniformis* MTCC 6824 was chromatographically purified to homogeneity by ammonium sulphate (70%) and ethanol/ether (1:1), followed by anion exchange and gel exclusion chromatography using Tris-HCl buffer (pH 8.0). The purified lipase was employed to evaluate its catalytic efficiency for hydrolysis reaction to enrich Δ 5-PUFAs, viz., arachidonic acid (AA) and eicosapentaenoic acid (EPA) content of triglycerides in sardine oil. Sardine oil was hydrolyzed by purified lipase (300 LU) under inert atmosphere of N₂. The glycerides were recovered by extraction with hexane. Pure triglycerides from the hexane residue were obtained by alumina column chromatography using n-hexane/diethyl ether (90/10, v/v) as eluant. The free fatty acids were further concentrated by urea-fractionation at different temperatures 4°C and urea/fatty acid ratio of 4:1. Free fatty acids were derivatized to their methyl esters by trans-esterification reaction for gas liquid chromatographic (GLC) and gas chromatographic-mass spectroscopic (GC/MS) analysis.

The lipase was purified from 48h *Bacillus licheniformis* MTCC 6824 culture supernatant with a

specific activity of 520.28 LU/mg. Lipase exhibited optimum activity at 45°C and pH 8.0. The enzyme exhibited 81.9% of the residual activity after 60 min of incubation at 45°C, and 69.3% after 2 h. Polyhydric alcohol, sorbitol was found to be an effective stabilizer of the enzyme, with about 98.6% of residual activity after 45 min, and 81.2% after 2 h of incubation. A combination of Ca²⁺ and sorbitol induced a synergistic effect on the activity of lipase with a significantly high residual activity (100%) even after 45 min, as compared to 91.5% when incubated with Ca²⁺ alone. The enzyme exhibited hydrolytic resistance towards ester bonds of Δ 5-PUFAs with respect to the presence of a Δ 5 olefinic double bond as compared with those of other fatty acids, and proved effective for increasing the concentration of EPA and AA from sardine oil. Utilizing this fatty acid specificity, EPA and AA from sardine oil were enriched by lipase-mediated hydrolysis followed by urea-fractionation at 4°C. The purified lipase produced highest degree of hydrolysis for SFAs and MUFAs (81.5 and 72.3% from their initial content in sardine oil) after 9 h. Lipase catalyzed hydrolysis of sardine oil followed by urea adduction with methanol provided free fatty acids with 55.38% EPA and 5.80% AA, respectively after complexation of saturated and less unsaturated fatty acids. Combination of enzymatic hydrolysis with urea complexation is a promising method to obtain highly concentrated EPA and AA from sardine oil.