Value enhancement of crude fish oil (capelin oil) using pancreatic lipase

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Sources of wax esters for commercial use are constantly being sought. Capelin oil, an inexpensive and abundant fish oil, frequently contains significant levels of wax esters derived from its zooplankton diet. The separation of wax esters from the triacylglycerols which comprise the bulk of the capelin oil is, however, difficult by conventional chemical means. Since free fatty acids are more easily separated than triacylglycerols from wax esters, pancreatic lipase was examined as a means of hydrolysing the triacylglycerols to free fatty acids while leaving the wax esters intact.

Assays of total volume 3.0 ml were performed in which crude capelin oil comprising 85% triacylglycerols and 12% wax esters was incubated with porcine pancreatic lipase. The concentration of components was varied to establish optimum conditions. After acidification of the lipid extract, with 0.5 M-Tris/HCl, pH 7.7. The rate of lipolysis was dependent on temperature up to 30°C. The extent of lipolysis was dependent on the volume of oil present in the assay medium. At contents of oil of less than 9%, hydrolysis of wax esters occurred as measured by the appearance of free fatty alcohols in the lipid extract. With levels in excess of 9%, not all the triacylglycerols were hydrolysed within the assay time.

To separate wax esters from the free fatty acids, lipid extracted from the lipase digestion mixture was applied to a glass column packed with alkalized silica and the column eluted sequentially with the following solvent systems: (1) 2 bed volumes 5% (v/v) diethyl ether in hexane; (2) 4 bed volumes 0.3% (v/v) formic acid in 15% (v/v) diethyl ether in hexane; (3) 2 bed volumes diethyl ether and (4) 2 bed volumes chloroform. Fractions corresponding to the different solvent systems were collected and the lipid weighed after removal of solvent. Component classes were quantified by t.l.c./f.i.d. Fraction 1 contained pure wax esters (Table 1). G.l.c. analysis showed that 20:1 (n = 9) and 22:1 (n = 11) together comprised 68% of the wax ester fatty alcohols and that monoenes and polyunsaturates accounted for 55% and 33% respectively of the fatty acid moieties. Only traces of other lipids, usually triacylglycerols were occasionally observed in this fraction. Fractons 2 and 3 contained pure monoacylglycerols. Fraction 4 contained monoacylglycerols, 50% of the monoacylglycerols were polyunsaturated, predominantly 20:5 (n = 3) and 22:6 (n = 3). The triacylglycerols of the starting oil contained 31% polyunsaturates.

The use of lipases in lipid biotechnology has mainly been associated with vegetable or microbial lipids (Rattray, 1984).

Abbreviation used: t.l.c./f.i.d., t.l.c. with flame ionization detection.

Table 1. Lipid composition of fractions prepared from capelin oil treated with pancreatic lipase

<table>
<thead>
<tr>
<th>Fraction no.</th>
<th>Total lipid recovered (%)</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Wax esters</td>
</tr>
<tr>
<td>1</td>
<td>12.0 ± 0.8</td>
<td>98.4 ± 0.7</td>
</tr>
<tr>
<td>2</td>
<td>35.7 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>48.0 ± 6.5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4.3 ± 1.7</td>
<td></td>
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</table>

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The present procedure uses a lipase to derive wax esters of high commercial value from an inexpensive fish oil. The monoacylglycerol may be of potential use as a starting substrate for the synthesis of tailored triacylglycerols for use in human health care products.

The saturated-fat-fed hamster as a model of atherosclerosis

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The need for an animal model of atherosclerosis is fundamental to the processes of understanding and treating this all too common cause of death in the western world. Feeding animals with a cholesterol-supplemented diet has not proved to be a predictive model for drug treatment of atherosclerosis, but the arterial lesions produced by this technique have been shown to be similar to those found in man (Nistor et al., 1987). Spady & Dietschy (1988) have shown that feeding hamsters with a diet containing 20% (w/w) coconut oil with a small supplement of cholesterol (0.1% w/w) produces a substantial hypercholesterolaemia. Deposition of cholesterol, as the ester, in the artery wall is presumably dependent on the action of acyl-CoA: cholesterol acyltransferase (ACAT). It is possible that prolonged exposure of the artery to high plasma cholesterol levels causes an increase in ACAT activity in the artery wall. We have compared the effects of inducing hypercholesterolaemia by feeding hamsters with 20% coconut oil in a diet already containing 0.1% cholesterol with those found after feeding a diet supplemented with 2% cholesterol. Plasma cholesterol, ACAT and 3-hydroxy-3-methylglutanyl (HMG)-CoA reductase activities in both liver and intestinal cells, and ACAT activity in superficial aorta were measured after 1, 2 and 4 weeks of feeding.

Male Syrian hamsters weighing 100–125 g at the start of the experiment were used, and weighed regularly throughout the experiment. After the animals had been fed on the diet for the required time, they were anaesthetized and the liver, intestine, aorta and a blood sample were taken for analysis. ACAT activity was assayed in liver microsomes and in homogenates of intestinal cells by measuring the incorporation of [14C]cholesterol into [14C]cholesteryl oleate as previously described (Suckling et al., 1982). The aorta was prepared for assay by removing the adventitia and homogenizing the intimal layers in a Tris/HCl buffer with a glass/glass homogenizer. The homogenate was then incubated with [14C]oleoyl coenzyme A and the [14C]cholesterol oleate formed was determined (our unpublished work). HMG-CoA reductase activity was quantified in liver microsomes and intestinal cell homogenates by the incorporation of [14C]HMG-CoA into [14C]mevalonic acid as previously described (Ingebritsen & Gibson, 1981). The products of these assays were separated from the substrate on silica t.l.c. plates, and quantified by scraping the appropriate peak of radioactivity for liquid scintillation counting. The plasma lipoproteins were fractionated by ultracentrifugation, and cholesterol concentrations in the fractions were determined using an enzymatic assay based on cholesterol oxidase (CHOD-iodide kits (Merck)).

It was found that feeding a cholesterol-enriched diet caused a rapid increase in plasma cholesterol levels from control levels of 111 mg/dl ± 20 (mean ± s.d., n = 6) to 341 ± 68 (n = 6) after 4 weeks feeding. The liver and intestinal cell HMG-CoA reductase activity was reduced and ACAT activity in these tissues was elevated. Saturated-fat feeding also caused a hypercholesterolaemia that peaked within 1 week of feeding at 235 mg/dl ± 65 (n = 4), gradually falling to 157 mg/dl ± 18 (n = 4) after 4 weeks of feeding. However, enzyme activities in liver and intestinal cells presented a different picture. Intestinal HMG-CoA reductase activity was slightly elevated, possibly in an attempt to synthesize sufficient cholesterol to allow the transportation of the large amounts of triacylglycerol being absorbed from the gut. ACAT activity in the liver and intestinal cells was greatly reduced, results consistent with those obtained by Spady & Dietschy (1988).

Increases in plasma low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol concentrations were found as a result of feeding either a cholesterol-enriched diet or a diet containing 20% coconut oil. There was a highly statistically significant correlation between plasma LDL cholesterol concentration and aorta ACAT activity, demonstrating that elevation of plasma LDL cholesterol levels can stimulate the deposition of cholesterol in the arterial wall, and that the source of that cholesterol, be it dietary or endogenously synthesized, is irrelevant. These data also demonstrate that the uptake mechanisms are not saturated at plasma cholesterol concentrations well above normal levels. We have also confirmed the findings of Spady & Dietschy (1988) that the cholesterol in the plasma of saturated-fat fed hamsters is not absorbed by the liver. The mechanism by which saturated fat feeding causes a hypercholesterolaemia is not clear, though Spady & Dietschy (1988) have suggested that down-regulation of LDL receptors in the liver would cause cholesterol secreted into the plasma from the liver to accumulate and so increase plasma cholesterol levels.

These data demonstrate that ACAT activity in the arterial wall is sensitive to changes in plasma LDL cholesterol concentrations, and that studying changes in ACAT activity in the aorta may be a useful indicator of the early stages of atheroma formation.

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REFERENCES


Abbreviations used: ACAT, acyl-CoA: cholesterol acyltransferase; LDL, low-density lipoprotein; HDL, high-density lipoprotein.