**PREPARATION OF HEPTADECENOCOIC ACID**

constant weight.

**RESULTS AND DISCUSSION**

The oil values determined by the oil signal and the solid-liquid signal methods, moisture content, and F-factor for each sun-dried sample of mustard, sunflower, and soybean have been given in Table I. The mean F-factors of these crops for their sun-dried seeds are 3.22, 2.87 and 2.98, respectively. The correlation in oil values determined by the two methods is 98.8% for mustard, 94.5% for sunflower, and 93.1% for soybean. It might be possible to improve the correlations for sunflower and soybean which have much larger seeds than mustard by analyzing larger samples of ca. 5 g in weight which would provide a more representative sample. This could be achieved by using an NMR probe head with a larger volume over which the RF and the magnetic fields are constant.

In the present experiment, the requirement of the representative sample has been fully met for mustard crop by using ca. 2.5 g seeds. The agreement between the oil values obtained by the two methods is very good for mustard, which means that the solid phase T2, moisture content, and proton density difference between the two phases of the seeds do not change significantly from sample to sample. The good agreement between the methods shows that it is possible to determine oil in seeds by pulsed NMR without weighing sun-dried seeds in plant breeding.

**REFERENCES**


[Received September 4, 1979]

---

**Preparation of Heptadecenoic Acid from Candida tropicalis Yeast**

D. BAUCHART and B. AUROUSSEAU, Laboratoire d'Etude du Metabolisme Energetique, I.N.R.A.-C.R.Z.V.-THEIX, 63110 Beaumont, France

**ABSTRACT**

In this paper a method is described for preparing 10 g or more of heptadecenoic acid (C17:1n8) of 99 p.100 purity from Candida tropicalis yeast. Three cycles of treatment, based on crystallization techniques, were used successively: (1) Crystallization of fatty acids (in free form) from acetone at -25 C induced the elimination of most of the saturated fatty acids, and at -60 C, of all of the polyunsaturated acids. (2) Inclusion formation of fatty acids (as methyl esters) with urea at hC induced the removal of all of the remaining saturated methyl esters and most of methyl oleate. (3) Crystallization of fatty acid methyl esters from acetone at -60 C removed almost all the remaining monounsaturated methyl esters (methyl palmitoleate and methyl oleate). Total efficiency of the preparation was about 17 p.100.

**INTRODUCTION**

Thorough studies of lipid metabolism involve qualitative and quantitative analyses of lipid classes and their fatty acids in biological samples. Among the different dosage methods, the addition of internal standards (free or esterified fatty acids) in the samples has the following advantages: sensitivity, specificity, and efficiency (simultaneous quantification of several kinds of lipids).

Among all the natural fatty acids readily available, heptadecenoic acid (C17:1n8) is the one that best possesses all the features which an internal standard should have: scarcity in most animal tissues, length of the carbon chain close to that of the main fatty acids in the biological samples, and easy usage in organic synthesis of esters (triacylglycerides, phospholipids, and cholesterol esters). Grown on hydrocarbons, Candida tropicalis yeast are rich in this acid (27.9 p.100 of total fatty acids). They are therefore an excellent biological source for preparing highly pure heptadecenoic acid.

Fractional distillation, often used in the past, could alter the structure of the fatty acid. Zhukov and Vereshchagin (1) avoided this problem and used preparative gas chromatography. Nevertheless, the authors worked out only some hundred milligrams of pure heptadecenoic acid, while a specialized, expensive apparatus would have been required for the preparation of several grams of this acid needed for our studies.

It is possible to separate great amounts of fatty acids into classes in a single step according to the degree of unsaturation with a simple liquid chromatography device. Florisil columns or ion exchange resins columns impregnated with silver nitrate have been used respectively by Anderson and Hollenbach (2) and Willner (3), and Wuster et al. (4) and Emken et al. (5). This technique, whose various uses and limits have been described by Morris (6), has been applied particularly to purify cis-olefins from trans-isomers by De Vries (7) and Emken et al. (5). It is, however, expensive, and its use is especially valuable for the purification of the polyunsaturated fatty acids.

Fractional crystallization techniques, based on the differences in physical properties of fatty acids, allow a greater field of purification possibilities. They usually do not cause any damage to fatty acids, are cheap and allow great quantities of pure fatty acids to be extracted. Brown (8) and Brown and Kolb (9), on the one hand, described the potentially numerous applications of low temperature fractional crystallization from solvents, and Schlenk (10) and Iverson and Weik (11), on the other hand, showed the advantages of the formation of inclusion complexes with urea. In both cases, the purification of a given fatty acid can be obtained only after several successive steps in the same treatment. Similarly, several authors have worked out methods of preparing highly pure fatty acids (99 p.100 pure or more) based on the use of either one of these two techniques or a combination of both. The raw sources used have been from either vegetable or animal origin: purification of oleic acid from olive oil by Swern and Parker (12), Keppler et al. (13), Rubin and Paisley (14), Fremont and Gazzelin (15), purification of linoleic acid from safflower oil by Brown (8) and Keppler et al. (13), or from corn oil by Schlenk and Holman.

JAOCs March 1980 / 121
(16); purification of arachidonic acid from pork liver by Privett et al. (17), purification of pentadecanoic acid from tuna oil by a combination of fractional crystallization and preparative gas chromatography by Stoffel and Ahrens (18), or from menhaden oil by a combination of methods of fractionation and liquid-liquid partition chromatography by Privett and Nickell (19).

The aim of the present work is to describe a method of preparing large quantities (17 g) of heptadecenoic acid (C17:1٠8) from the total lipids of Candida tropicalis yeast grown on hydrocarbons. This method is based on a combined use of low temperature fractional crystallization from acetone, on the one hand (free fatty acids or methyl esters), and fractional inclusion complex formation with urea, on the other hand (fatty acids as methyl esters).

MATERIALS AND METHODS

Preparation of Fatty Acids and Methyl Esters

Total lipids were extracted as described by Folch et al. (20), from four samples of 1 kg of Candida tropicalis yeast, collected after growth on a mixture of C13 to C18 hydrocarbons. They were saponified by a 10 p.100 solution of alcoholic KOH overnight at 20 C. After extracting unsaponifiable materials with hexane, fatty acids were released by the addition of HCl 6N and were extracted with hexane. They were washed with distilled water until neutral and dried over pure anhydrous sodium sulfate. Fatty acids were methylated by boiling and refluxing the solvent for 30 min with 3 p.100 methanol HCl.

Fatty Acids Crystallization

From acetone. The following concentrations were used: 5 p.100 for fatty acids in free form and 10 p. 100 for fatty acids as methyl esters. The solutions were kept at -25 C or -60 C in an ultra Kryostat (UK 80 DW Laua) in a methanolic bath, for 6 hr, so crystallization could take place. Crystals (C) were separated from the filtrate (F) by filtration under vacuum with a sintered glass funnel no 0 previously chilled at -25 C. Crystals gathered on the funnel were dissolved in chloroform. Fatty acids or their methyl esters were then recovered from the crystals or the filtrate after the solvent had been withdrawn by evaporation under vacuum.

With urea and methanol. The crystallization of fatty acids (as methyl esters) with urea was generally carried out under the following conditions: (a) 1 part (g) of methyl esters, (b) 10 volumes (ml) of methanol, and (c) 1 part (g) of urea.

Nevertheless, when small amounts of saturated fatty acids were present in the mixture, the quantity of urea was increased two- or threefold. Methyl esters were rendered soluble in the methanol in a glass Pyrex flask, and urea was then added to the solution. Its complete dissolution was obtained by heating the solution with the help of an electric hot plate. Afterwards, the solution was allowed to cool at room temperature and then transferred to a cold room at 4 C overnight. It was filtered under mild vacuum through a sintered glass funnel no 0 kept at 4 C.

Methyl esters were systematically reextracted from filtrates and crystals. First of all, methanol was removed by vacuum evaporation, and the urea of the remainder was solubilized in a boiling 1 p.100 HCl solution. Methyl esters were extracted with hexane and weighed, and their composition was studied by gas chromatography.

Analytical Techniques

The C17:1٠8 content of every successive fraction was determined by gas chromatography of their methyl esters on an open tubular column of stainless steel impregnated with Carbowax 20M-terephthalic acid (100 m long and 0.5 mm in diameter). The device consisted of a Packard Type 427 chromatograph fitted with a FID detector and connected to an electronic integrator LTI leap 5 and a Houston Omniscribe potentiometric recorder. The following procedure was adopted: oven temperature, 190 C; detector and injector temperature, 240 C. The carrier gas was nitrogen “u” filtered on an O2 and H2 absorber Oxysorb (Airgaz). Its flow rate was 0.35 ml/mn corresponding to, in our operative conditions, a pressure of 0.4 bar. Fatty acid identifications were obtained by calculating their corresponding equivalent chain length (ECL) values from retention times supplied by the electronic integrator and then, by comparing these values either to those of ECL tables from Flanzy et al. (21) or to those worked out in our laboratory from standard commercial fatty acids (Nu Chek Prep) under similar operative conditions. The structure of the last purified fraction of methyl heptadecenoic (fraction C7****) was also checked by the analysis of its infrared spectrum, supplied with an infrared Beckman model 420 spectrophotometer with a solvent-free compound in a 0.1 mm cell. The purity control was determined by recording the nuclear magnetic resonance (NMR) spectrum with a deuterochloroform solution on a Varian T60 spectrometer operating at 60 MHZ.

RESULTS AND DISCUSSION

The total lipid composition (13.8 p.100 of dry matter) of Candida tropicalis yeast is indicated in Table I. Their phospholipid content was 70 p.100, which explained their low fatty acid content (8.9 p.100). The fatty acids composition of both classes of lipid yeast (phospholipids and triglycerides, Table I) was determined. Since they were very similar, there was no need to separate the two classes. Extraction yielded 346 g fatty acids from 4 kg of yeast. According to its composition, the initial mixture included 96.5 g heptadecenoic acid (C17:1٠8). Apart from this acid (27.9 p.100), total lipids included 45.7 p.100

| TABLE I |
| Extraction of Total Fatty Acids of Candida tropicalis Yeast: Some Characteristics of the Fractions at the Main Steps of Preparation |

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Centesimal composition (g)</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast (dry matter, p.100 of fresh weight)</td>
<td>96.9</td>
<td>4000</td>
</tr>
<tr>
<td>Total lipids (p.100 of dry matter)</td>
<td>13.8</td>
<td>70</td>
</tr>
<tr>
<td>Phospholipids (p.100 of total lipids)</td>
<td>8.9</td>
<td>346</td>
</tr>
<tr>
<td>Triglycerides (p.100 of total lipids)</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Fatty acids (p.100 of dry matter)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

122 / JAOCS March 1980