ISOLATION OF LIPID PARTICLES FROM BAKER'S YEAST

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1. Introduction

The presence of lipid particles in yeast cells has been demonstrated by light as well as by electron microscopy [1], but the more precise nature of the lipid is unknown. The present letter describes the isolation and subsequent chemical analysis of lipid particles from baker's yeast (*Saccharomyces cerevisiae*). The results show that triacylglycerols and sterol esters are the major components of the lipid and account for 90–95% of the total particle content.

2. Isolation of lipid particles

Homogenates of yeast cells were prepared from 400 *g* of freshly harvested, packed cells as described by Schatz [2] except that 0.4% (w/v) bovine serum albumine was included in the mannitol–Tris–EDTA medium (MTEB-medium) and that pH was readjusted to pH 7.4 after homogenization of each batch of cell suspension. The final homogenate (900 ml) was centrifuged 15 min at 13 000 rpm in the GSA rotor of the Sorval RC2-B centrifuge, and the lipid enriched upper half of the supernatant was siphoned off. The remaining supernatant and sediment were removed and lipid adhering to the wall of the centrifuge bottles was suspended in the lipid enriched supernatant. The volume of this lipid suspension was reduced to about 20 ml by two further centrifugations: One at 13 000 rpm for 15 min after which again the upper half of the supernatant was collected, and one at 20 000 rpm for 20 min performed in 50 ml tubes in the SS-34 rotor. After the latter centrifugation the lipid was packed at the top of the tubes and was collected by suspension in the small volume of supernatant left unfrozen after immersion of the tubes in a dry-ice–ethanol mixture to about 1 cm below the surface of the tube content.

This crude preparation of particles was purified by flotation through MTE medium (MTEB medium minus bovine serum albumine). The crude particle suspension was mixed with an equal volume of 50% (w/v) sucrose and layered below 2 volumes of MTE medium in 15 ml capacity tubes. The tubes were centrifuged in 15 min at 20 000 rpm in the SS-34 rotor, and the lipid was collected after freezing of the tube content as described above. This washing procedure was repeated once, and the particles were suspended in a known volume of MTE medium and stored at 4°C.

The yield of particles from 400 g of packed cells varied between 150 and 200 mg dry weight.

3. Electron microscopy

The lipid particles collected at the top of the washing medium after the last washing were very easily dispersed. However, it was possible with caution to transfer small flakes of lipid on a spatula to another tube for fixation. The flakes were fixed for 24 hr in 3% glutaraldehyde buffered with 0.1 M cacodylate buffer pH 6.8 and then for 2 hr in 2% osmium tetroxide in the same buffer. Samples of intact cells were fixed in 4% glutaraldehyde for 48 hr followed by fixation in 2% osmium tetroxide for 4 hr. Dehydration and embedding in Araldite followed conventional procedures without special measures to increase retention of lipid. Sections

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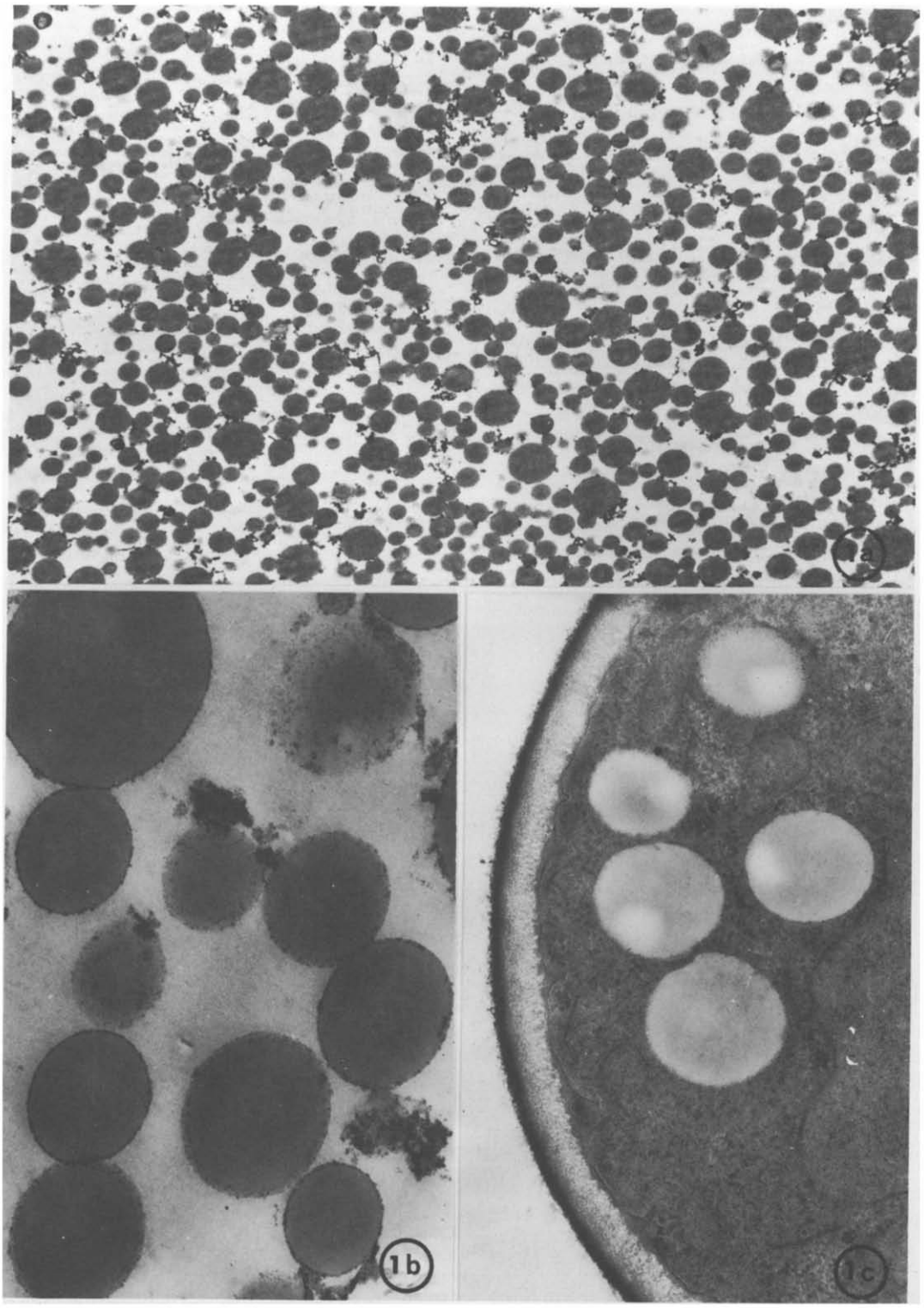

Fig. 1. Yeast lipid particles: (1a) isolated × 9000; (1b) isolated × 41 000; (1c) in situ × 41 000.

were contrasted with uranyl acetate and/or lead acetate prior to viewing in a Philips 300 electron microscope.

Electron micrographs of isolated particles are presented in fig. 1a and b. There is unconspicious contamination with non-lipid material. The diameters of the particles range from 0.3–1 μm for the majority of the particles, but some of the isolated particles are larger than observed in intact cells, and some coalescence of particles may therefore have taken place during homogenization and separation. The particles appear to be egg-shaped or globular and are of a rather homogeneous density. A thin layer of a more densely stained material, without any indication of a trilaminar membrane structure coats the surface of the particles. An electron micrograph of lipid particles in situ is presented in fig. 1c. The resemblance with the isolated particles is evident with respect to size and shape, but there is a marked difference in the density of staining. This, however, most probably reflects well known difficulties encountered in obtaining a satisfactory osmium fixation of intact yeast cells.

4. Chemical analysis

The protein content of the particles was determined ad modum. Lowry [3] except that lipid was removed (by extraction with chloroform) immediately before the absorbance of the colour complex was read. The protein content varied from 10–30% in the crude preparations and was reduced to an average of 6% in the purified preparations (table 1). The sum of protein and lipid agreed with dry weight determinations within a few per cent.

Total lipid was extracted from the suspensions of purified particles by two extractions with 20 volumes of chloroform: methanol (2:1). The combined extracts were washed with 0.2 volume of 0.9% (w/v) aqueous NaCl and twice with 0.2 volume of distilled water. After drying with anhydrous Na_2SO_4 the solvents were removed by evaporation under N_2 and the lipid was redissolved in a known volume of chloroform and stored under N_2 at 4°C. Aliquots were evaporated to dryness and weighed on a Cahn electrobalance. The average yield of lipid obtained in 5 preparations from 400 g cells was 160 mg (table 1).

Table 1
Composition of lipid particles from baker's yeast*

	mg per 100 mg Dry weight of particles	mg per 100 mg Total lipid
Protein	6 ± 0.6	
Lipid	94 ± 10	
Triacylglycerols		47 ± 2
Sterol esters		44 ± 2
Phospholipid		0.5 ± 0.1
Free fatty acids		0.5 ± 0.1

* Average from 5 preparations ± standard error of the mean.

Lipid classes in extracts of total lipid were separated by TLC developed with light petroleum (b.v. range 60–80°C)–diethyl ether–acetic acid (85:15:1, v/v/v) and the following components were identified: triacylglycerols, sterol esters, phospholipids and free fatty acids. It was not possible to detect mono- or diacyl-glycerols. The amount of triacylglycerols were determined by enzymic glycerol analysis [4] of hydrolyzed samples of total lipid, ignoring the small amounts of glycerol from phospholipid. Sterol esters were estimated by sterol analysis [5] of lipid eluted from TLC spots running as ergesterol ester and hydrolyzed. Phospholipid was calculated from values of total lipid P [6] assuming a 4% content of P in phospholipids. Free fatty acids were determined according to Duncombe [7]. The results are summarized in table 1. They show that the lipid consists of about equal amounts of triacylglycerols and sterol esters with less than 1% of phospholipid and free fatty acids.

The fatty acid composition of triacylgycerols and sterol esters was analyzed by gas chromatography. The lipids were eluted from TLC and treated with 5% (w/v) HCl in anhydrous methanol to give methyl-esters. Table 2 shows that monoenoic acids with chain lengths of 16 and 18 carbon atoms were the major constituents both in triacylglycerols and in sterol esters, and that polyenoic acids were more abundant in sterol esters than in triacylglycerols.

Table 2
Fatty acid composition of triacylglycerols and sterol esters in lipid particles

	Percent of total methylesters									
	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:0
Triacyl-glycerols	–	6	27	6	38	12	5	2	2	2
Sterol esters	–	4	23	3	33	22	9	1	2	3

5. Discussion

The isolation and subsequent analysis of particles from yeast cells generally described as lipid granules, droplets or spherosomes have confirmed their lipid nature and identified the major components as triacylglycerols and sterol esters.

The electron microscopic appearance of the particles is similar to that of a number of lipid particles which in recent years have been isolated from plant and animal material under a variety of names: spherosomes from peanuts [8], 'simple' lipid vesicles from bush beans [9,10], 'mature' oil droplets from castor beans [11] and lipid droplets from liver [12] and kidney medulla [13,14]. In all these particles triacylglycerols have been found to account for about 90% of the particle content*, and it is generally accepted that these particles serve as a store of fatty acids for energy production. The presence in the yeast lipid particles of equal amounts of triacylglycerols and sterol esters and the more unsaturated character of the fatty acids in the sterol esters, may indicate that these particles serve as a store not only for energy production but also for membrane synthesis.

Acknowledgements

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* A sterol ester content of appr. 30% in lipid droplets from kidney medulla [13] has been found to be an artifact. (I. Bojesen, personal communication).

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