Conditions of Isolation of Lipids from Yeast

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Received December 20, 1968

In revised form April 16, 1969

A comparison of the efficiency of several methods for lipid extraction from yeast was made. None of the tested methods enables to isolate total lipids by one step extraction. A two step extraction procedure enabling quantitative determination of total yeast lipids was elaborated.

The study of lipid composition of yeast is conditioned by quantitative isolation and perfect fractionation of lipids. A survey of methods for isolation of yeast lipids was written by *Hoppe* [1]. Lipids are generally extracted with a mixture of organic solvents either directly [2—4] or after previous saponification [5—8].

Harrison and *Trevelyan* [9] have recently demonstrated that some fractions of lipids could be extracted completely only when yeast cells were mechanically disintegrated. The mechanical disintegration of yeast is associated, however, with numerous difficulties. The purpose of this work was therefore to reexamine the conditions of lipid extraction from unbroken yeast cells, and to demonstrate the insufficiency of some known extraction procedures, and to find a simple method for quantitative determination of lipid composition of yeast omitting the mechanical disintegration of cells.

Experimental

Reagents

All chemicals used in this study were purchased from Lachema, Brno. Organic solvents were always freshly distilled.

Microorganism and its cultivation

The strain *Saccharomyces cerevisiae DTXII* was grown anaerobically in a synthetic medium at 30°C [15]. The culture having reached stationary phase was cooled (0°C) and cells were centrifuged off, washed four times with ice-cold water being bubbled with argon and then lyophilized.

Lipid analysis

To find good conditions for lipid extraction from lyophilized yeast, extraction procedures according to *Folch* [10], *Letters* [11], *Katchmann* [2], *Kaufer* [12] and *Klein* [6] were applied. The extraction was also done in an apparatus similar to Soxhlet one.

As concerns phospholipids, a slightly modified procedure of *Kaufer* [12] was found to be the most effective and very suitable for a serie of analysis. Up to 100 mg of lyophilized

yeast were suspended in 5 ml of 10% trichloroacetic acid. The suspension was centrifuged after 30 minutes. The sediment was resuspended in 7 ml of methanol, then heated for 15 minutes at 55°C in stoppered tubes. After cooling 14 ml of chloroform were added and the mixture was vigorously shaken for several minutes and left to stand overnight at room temperature. In the morning the mixture was centrifuged and the resulting sediment was washed with chloroform—methanol (2 : 1, v/v). The two supernatants were concentrated under argon and washed according to *Folch* [10]. Phospholipids were estimated on the basis of inorganic phosphorus determination [13] in the mineralized sample [2].

Maximum yields of saponifiable lipids as well as of total fatty acids were obtained by the method of *Klein* [6] modified in a few details as follows: 100 mg of lyophilized yeast were suspended in 1 ml of saturated KOH followed by addition of 3 ml of ethanol. The samples were placed on an 80°C bath. After a few minutes 4 ml of ethanol were added and the saponification continued for 4 hours. Evaporated ethanol during this treatment was occasionally replaced. After the saponification, the cells were centrifuged off and the quantitatively transferred supernatant was concentrated to a small volume. To 2 ml of the sample 9 ml of water were added and the unsaponifiable lipids were removed by three successive extractions with 10 ml of petroleum ether. The rest of the saponified extract was treated with H_2SO_4 and again extracted three times with 10 ml of petroleum ether to give a fraction representing total fatty acids. Concentrated extracts were washed [10] and total unsaponifiable lipids and total fatty acids were estimated photometrically with dichromate [14]. The saponification and the concentration of samples were carried out in the argon atmosphere.

Results and Discussion

All experimental results indicate that the extraction process is the decisive step for quantitative analysis of yeast lipids. It was found that a simple application of organic solvent mixtures used for lipid extraction from mammal cells or subcellular particles is not sufficient in the case of whole yeast cells (Fig. 1, extraction procedures No. *1—5*). Thus the results obtained by similar methods should be interpreted more carefully.

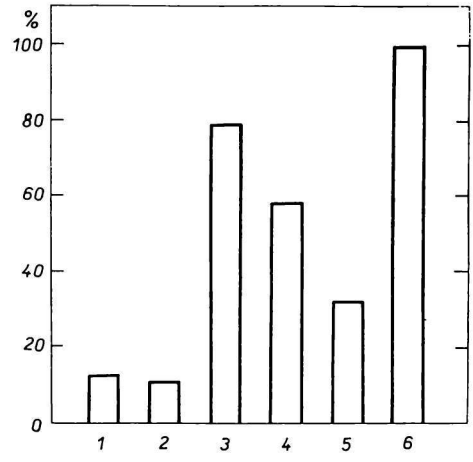

Fig. 1. Comparison of the amount of total yeast lipids extracted by various methods. *1.* using an extraction apparatus and chloroform—methanol (2 : 1, v/v) as the solvent mixture at the temperature near the boiling point of the mixture for 24 hours; *2.* according to *Letters* [11]; *3. Katchmann* [2]; *4. Kaufer* [12]; *5. Folch* [10]; *6. Klein* [6].

As seen in Fig. 2, the best extraction of phospholipids was achieved by the method of *Kaufer* [12] originally used for *E. coli* as well as by a similar method of *Katchmann* [2]. The cells treated with trichloroacetic acid were extracted with methanol and chloroform—methanol (2 : 1, v/v) in the former case, and with ethanol and ethanol—diethylether (3 : 1, v/v) in the latter one. While the phospholipid extraction was quite reliable, both above procedures were insufficient for quantitative extraction of total lipids. This may be due to the fact that some of the lipid fractions are covalently bound to cell structural components which are insoluble in organic solvents.

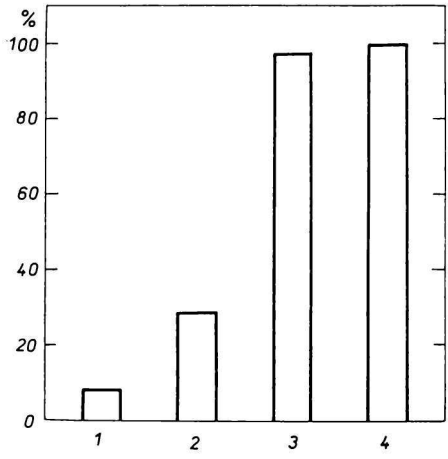

Fig. 2. Comparison of the amount of phospholipids extracted by various methods.
1. in an extraction apparatus as described under Fig. 1; *2.* according to *Letters* [11]; *3. Katchmann* [2]; *4. Kaufer* [12].

Relatively low yields of lipids were also obtained by method of *Folch* [10], *Letters* [11] or by using an extraction apparatus resembling the Soxhlet one. All these procedures are based on the direct extraction of untreated cells with the chloroform—methanol mixture.

The highest yields of unsaponifiable lipids and fatty acids were obtained only using more drastic treatment of cells which included the saponification with alcoholic KOH (Fig. 1, procedure No. *6*). This method afforded reproducible results with the relative deviation on the average 6.9%. Although this procedure has been successfully used for estimation of unsaponifiable lipids and fatty acids in yeast [6], it does not permit, however, to distinguish between free and esterified fatty acids and to determine phospholipids.

To obtain a complete picture of the quantitative composition of yeast lipids, it is necessary to carry out the extraction by two independant methods enabling the estimation of different lipid fractions separately. The analysis of phospholipids described in Experimental is quantitative with the relative deviation on the average 1%. The method is not pretentious and for a serie of analysis seems to be more suitable then the *Katchmann's* procedure [2]. The results attained by this method [15] are in good agreement with those of other authors [9, 16] who extracted phospholipids from mechanically disintegrated cells, but the values are higher than those reported by *Miyamoto* [4] using a repeated extraction of whole cells. Since it was

clearly shown that unsaponifiable lipids and fatty acids could be estimated in extracts only after forgoing saponification, the validity of results obtained by *Miyamoto* [4] is arguable.

I am indebted to Dr. L. Kováč for his valuable advice during this work.

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Translated by P. Biely