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Note

High-performance liquid chromatographic analysis of major carotenoids from *Rhodotorula glutinis*

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A potent intracellular lipid-producing yeast, *Rhodotorula glutinis*, produces carotenoid pigments mainly during the stationary phase of its growth and the characteristics of the carotenoid pigments have been well established and four major carotenoids, *i.e.*, torulene, torularhodin, β-carotene and γ-carotene were identified[1–3].

Until recently, column chromatography has been mainly used for the microbial carotenoids including this organism[2–5]. However, this technique has its limitations such as the large quantity of sample required, poor resolution, incomplete recovery and artifact production[6]. On the other hand, the use of high-performance liquid chromatography (HPLC) offers several advantages over classical chromatography including high effiency, selectivity, speed and mild conditions[7].

Although both plant carotenoids[8–12] and plasma carotenoids[13–15] were analyzed by HPLC, there are few reports on the HPLC analysis of microbial carotenoids except for a few examples[16]. Moreover, to our knowledge, no published methods can quantify all major carotenoids of *Rhodotorula glutinis* simultaneously in one HPLC step.

The purpose of this study was to develop a simple and rapid procedure for the analysis of major carotenoids from *Rhodotorula glutinis*.

EXPERIMENTAL

Microorganism

A strain of the yeast, *Rhodotorula glutinis* NRRL Y-1091, was used. It was maintained and cultured as described by Yoon and Rhee[1].

Samples and reagents

Pigment mixtures from *Rhodotorula glutinis* were prepared as described by Simpson *et al.*[17]. β-Carotene was obtained from Sigma. Other carotenoid standards were prepared by the procedure of Simpson *et al.*[17]. Each standard compound was identified by its position on the column, and by their light absorption curve[17]. Acetonitrile and tetrahydrofuran were of HPLC grade (Merck). All other reagents were of analytical grade (J. T. Baker).

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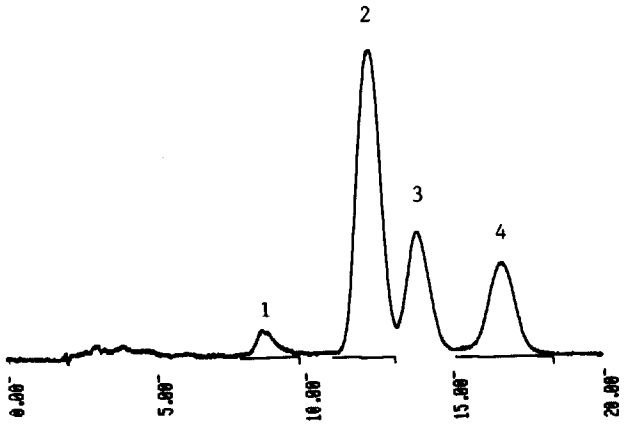

Fig. 1. HPLC chromatogram of carotenoid pigments from *Rhodotorula glutinis*. Conditions: Column, C_{18} Z-module; flow-rate, 2.00 ml/min; eluent, acetonitrile–tetrahydrofuran–water (50:38.5:11.5, v/v/v); detection, 436 nm; sensitivity, 0.05 a.u.f.s. Peaks: 1 = torularhodin; 2 = torulene; 3 = γ-carotene; 4 = β-carotene.

Chromatography

The carotenoid analyses were performed with a Waters Assoc. chromatograph which was equipped with a Waters Model 510 pump and connected to a Waters 740 data module. The column used was Z-module C_{18} and the operating conditions were as follows; elution solvent, acetonitrile–tetrahydrofuran–water 50:38.5:11.5, v/v/v); sample solvent, light petroleum (b.p. 30–70°C); flow-rate, 2.00 ml/min.

The absorption of the carotenoids was measured at 436 nm by a Waters Model 440 absorbance detector. The quantitative analysis was performed with the single-point calibration method using an external standard, the concentration of which was confirmed by a spectrophotometric measurement[17].

RESULTS AND DISCUSSION

Classical column chromatography for the separation of carotenoid components was accompanied by a time-consuming procedure involving saponification, washing, column separation, phase separation, drying and spectrophotometric measure-

TABLE I

AMOUNTS OF CAROTENOID COMPONENTS IN *R. GLUTINIS*

NA = Not available.

No. of injection	*μg Carotenoid component per g cell dry weight*			
	Torularhodin	*Torulene*	*γ-Carotene*	*β-Carotene*
1	1.13	40.73	13.91	21.34
2	1.15	43.70	14.74	21.46
3	0.91	44.01	14.56	NA

ment[2–5]. Compared to this method, pigment mixtures on the HPLC column could be satisfactorily separated in less than 20 min, as shown in Fig. 1. Since a reversed-phase column (C_{18}-Z-module) was used, the compounds were eluted in the order of decreasing polarity: torularhodin, torulene, γ-carotene, β-carotene. Each peak was identified by comparison of its retention time with that of the standard compound, and confirmed by the corresponding peak area, when co-injected with each standard compound. The purity of each standard compound was >95% as determined from the HPLC peak area. The chromatogram of pigment mixtures was reproducible. The reproducibility of the quantification of the carotenoid components is shown in Table I.

In conclusion, HPLC provides an efficient method for the separation and quantification of carotenoid pigments from *Rhodotorula glutinis*. Furthermore, the method may be extended for preparative separation.

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