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Short communication

Determination of thiamine in dried yeast by high-performance liquid chromatography using a clean-up column of CM-cellulose

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Abstract

Thiamine in dried yeast was cleaned up with a CM-cellulose column and determined by HPLC on a reversed-phase ODS column with sodium 1-octanesulfonate as an ion-pairing agent and UV detection. Thiamine and phenacetin as an internal standard were monitored with UV detection at 254 nm. The calibration graph was linear in the range 0.25–4 μg/ml of thiamine (as thiamine hydrochloride) with a correlation coefficient of 0.998. Intra- and inter-day variations of thiamine in dried yeast were 6.5 and 6.9%, respectively. Thiamine was recovered in good yield (97.8–105.7%, $n = 5$). By using this method, the thiamine content in dried yeast was found to be 138–169 μg/g. These results were in good agreement with those of the conventional thiochrome method.

Keywords: Sample preparation; Thiamine; Phenacetin; Vitamins

1. Introduction

Dried yeast is refined from beer brewing [1] and contains proteins, essential amino acids, vitamins and minerals [2]. Thiamine in dried yeast is an especially important compound from the viewpoint of dietetics. Most of the thiamine in living bodies and natural products exists as diphosphate, which works as a coenzyme of sugar metabolism system enzymes (active vitamin B_1).

Thiamine is converted into a highly fluorescent product, thiochrome, by chemical oxidation using potassium hexacyanoferrate (III) [3] or cyanogen bromide [4] in the presence of sodium hydroxide. Especially the latter method has been used for the conventional determination of thiamine in dried yeast because of its specificity and sensitivity [1]. However, the method requires a considerable amount of cyanogen bromide, which is very toxic to living bodies. Therefore, the conventional thiochrome method presents disposal problems to the environment.

From this reason, a method for the determination of thiamine in dried yeast without using this reagent was developed in our laboratory [5]. Thiamine was extracted from dried yeast extract with isobutanol containing sodium 1-octanesulfonate, and was determined by HPLC with UV detection. However, it was not as easy to evapo-

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rate to dryness isobutanol containing sodium 1-octanesulfonate.

The aim of this study was to develop an easier clean-up method for thiamine in dried yeast by using CM-cellulose and to apply this method to dried yeast. Also, the efficiency and reliability of this method were compared with those of the conventional thiochrome method [1].

2. Experimental

2.1. Materials and reagents

Thiamine hydrochloride was of Japanese Pharmacopoeia (JP) Standard grade, obtained from the National Institute of Hygienic Sciences [1]. Dried yeast (three lots) was obtained from Asahi Beer (Tokyo, Japan). Phenacetin was of JP grade from Maruishi Seiyaku (Osaka, Japan). Taka-diastase was of vitamin B_1 determination grade (100 units/g), purchased from Sankyo (Tokyo, Japan). Acetonitrile and sodium 1-octanesulfonate were of HPLC grade from Katayama Chemical (Osaka, Japan) and Regis Chemical (Morton Grove, IL, USA), respectively. CM-cellulose (CM-Cellulofine C-200, 44–105 μm) was purchased from Biochemical Industry (Tokyo, Japan). And other all solvents and chemicals were of analytical-reagent grade from Katayama Chemical.

2.2. Pretreatment of CM-cellulose

CM-cellulose does not physically adsorb thiamine. That is, CM-cellulose binds thiamine with only ionic bonds and it can be eluted perfectly. Also, the spherical type is convenient from the viewpoint of handling. Therefore, CM-Cellulofine (spherical type) was selected for purifying thiamine in dried yeast.

CM-cellulose was conditioned in a beaker by washing sequentially with 0.2 *M* hydrochloric acid, water, 0.2 *M* sodium hydroxide, water and 0.2 *M* hydrochloric acid. Finally, it was rinsed with water until its pH became neutral. A 2.5-ml volume of the conditioned CM-cellulose was packed into a chromatographic column (170 mm × 10 mm I.D.). It was washed with 0.3 *M* phosphoric acid and rinsed with water until its pH became neutral.

2.3. Sample preparation

The extraction of thiamine from dried yeast and the hydrolysis of thiamine phosphates with enzyme were carried out according to the conventional thiochrome method in the Japanese Pharmacopoeia [1]. The total thiamine content is considered to give an indication of pharmacological effects with regard to vitamins deficiency, etc. Therefore, the thiamine content in dried yeast was determined as total thiamine after thiamine phosphates had been hydrolysed with Taka-diastase.

Thiamine in 1 g of dried yeast was extracted with 1 ml of 10% (w/v) hydrochloric acid and 80 ml of water at 80–85°C for 30 min by frequent shaking. After the extract had been cooled, it was diluted to 100 ml with water and centrifuged for 10 min. To 4 ml of the supernatant were added 5 ml of 0.2 *M* acetic acid–sodium acetate buffer (pH 4.5) and 1 ml of Taka-diastase supernatant (30 mg/ml of 5 m*M* hydrochloric acid). The mixture was allowed to stand at 45–50°C for 3 h.

To the prepared clean-up column of CM-cellulose, 2 ml of this mixture were charged and eluted at a flow-rate of 0.5 ml/min. For the recovery test, 1 μg of thiamine hydrochloride was added to 2 ml of this mixture before cation exchange, then the column was washed twice with 10 ml of water at a flow-rate of 1 ml/min. Thiamine was eluted twice with 2.5 ml of 0.3 *M* phosphoric acid at a flow-rate of 0.5 ml/min and all the eluate was collected. To the eluate were added 1 ml of internal standard solution (1 μg/ml) and 0.01 g of sodium 1-octanesulfonate. The solution obtained was used as the sample solution.

Separately, to 1 ml of thiamine standard solution (1.5 μg/ml in 0.001 *M* hydrochloric acid) were added 1 ml of internal standard solution and 3 ml of mobile phase. The solution obtained was used as a standard solution. Volumes of 200

μl of the standard and sample solutions were injected into the HPLC system.

2.4. Instrumentation and chromatographic conditions

The HPLC system consisted of an LC-6A system with an SPD-6A (Shimadzu, Kyoto, Japan). Sample solutions were introduced manually with a syringe through a Rheodyne injector (Model 7161, 200-μl loop). The analytical columns were 150 mm × 4.6 mm I.D. stainless steel packed with 5-μm Nucleosil $5C_{18}$ (Chemco, Osaka, Japan) and Capcell-pak C_{18} (Shiseido, Tokyo, Japan). The peaks were monitored with UV detection at 254 nm and evaluated with a C-R5A integrator (Shimadzu). The mobile phase was 0.02 *M* phosphate buffer (pH 3.5) containing 0.2% (w/v) sodium 1-octanesulfonate–acetonitrile (4:1, v/v). The flow-rate was set at 1.0 ml/min and the column temperature was maintained at 40°C.

3. Results and discussion

3.1. Selection of eluate

A 1-μg amount of thiamine was charged to the column and was eluted with 0.1 *M* hydrochloric acid, 2 *M* acetic acid, 0.3 *M* phosphoric acid and 1 *M* sodium chloride. Elution with 0.3 *M* phosphoric acid gave the best recovery (ca. 100%).

3.2. Cation-exchange capacity of CM-cellulose

Volumes of 1, 1.5, 2, 2.5 and 3 ml of the solution after dried yeast extraction were charged into the CM-cellulose column to examine the cation-exchange capacity. A linear relationship was obtained between peak-area ratio and sample volume from 1 to 3 ml (Fig. 1); 2 ml was adopted as sample volume.

3.3. pH of the sample solution

For the study of the optimum pH of the sample solution, the behaviour of thiamine in the column was examined by using sample solutions with pH values of 2, 3, 4.5, 6, 7 and 8 adjusted with 1 *M* sodium hydroxide solution. The maximum amount eluted was obtained at pH 4.5, where the ionic bindings between the basic group of thiamine and the carboxymethyl groups of CM-cellulose are suggested to become maximum. Above pH 6 and below pH 3 thiamine was observed to adsorb imperfectly on the column. Hence the pH of the sample solution was adjusted to 4.5 by addition of acetic acid–sodium acetate buffer.

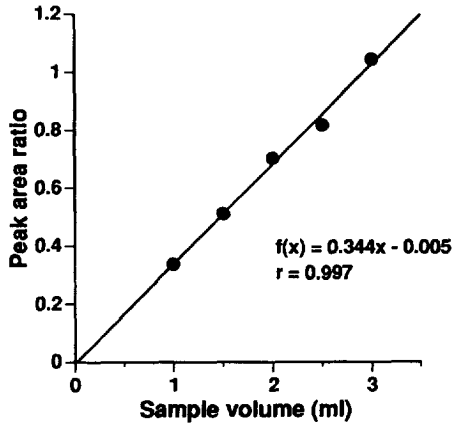

Fig. 1. Cation-exchange capacity of CM-cellulose.

3.4. HPLC conditions and internal standard

From viewpoints of peak shape and separation from impurities, phenacetin was selected as an internal standard. Typical chromatograms of thiamine in dried yeast by using Nucleosil and Capcell-pak columns were shown in Fig. 2. The resolutions of thiamine and phenacetin peaks using Nucleosil and Capcell-pak columns were approximately 9 and 6, respectively.

3.5. Linearity, recovery and precision

After the optimal HPLC and the pretreatment conditions were established as above, the linearity of response was examined. The curve of peak area ratios versus concentrations was obtained by analyzing standard solutions containing 0.25 to 4 μg/ml of thiamine hydrochloride and was found

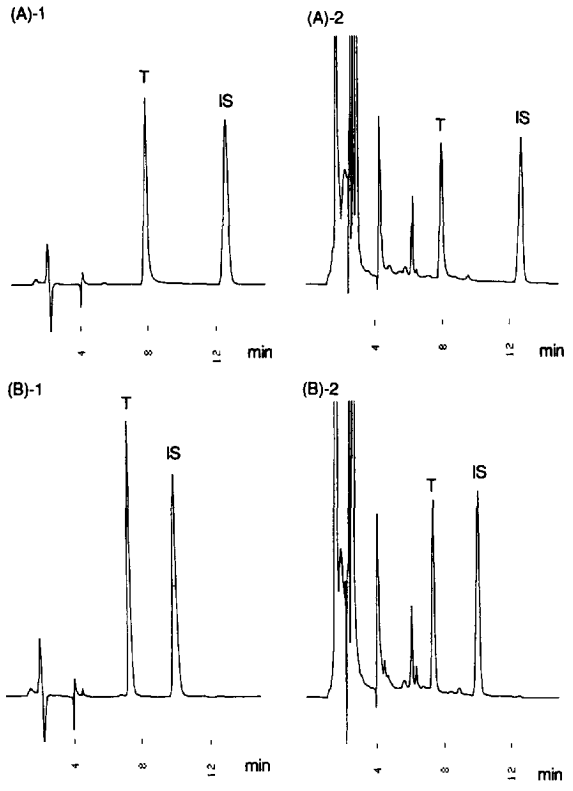

Fig. 2. Typical chromatograms of thiamine in dried yeast obtained by HPLC–UV using Nucleosil and Capcell-pak columns. (A)-1, standard (Nucleosil); (A)-2, sample (Nucleosil); (B)-1, standard (Capcell-pak); (B)-2, sample (Capcell-pak). Peaks: T = thiamine; IS = internal standard.

to be linear over the range with a correlation coefficient of 0.998. This range adequately is able to cover thiamine concentrations in dried yeast (25–400 μg/g of dried yeast).

The recovery by this method was in good yield of 97.8–105.7%, while that by thiochrome method was 88.4–99.6%. Coefficients of variation (%) of this HPLC and thiochrome methods were 3.2 and 4.4%, respectively. Intra- and inter-day variations of thiamine in dried yeast by HPLC were 6.5 and 6.9, respectively. Also, difference between Nucleosil and Capcell-pak columns was not very significant.

From these results, this HPLC method was considered to be able to replace thiochrome method.

3.6. Application

Thiamine contents in dried yeast (three lots) were assayed by this method using a Nucleosil $5C_{18}$ column and were found to be 138–169 μg/g. These results were in good agreement with those obtained by the conventional thiochrome method.

4. Conclusions

Pretreatment using a clean-up column of CM-cellulose is an easy and reliable technique for the determination of thiamine in dried yeast. This HPLC–UV method is considered to be able to replace the conventional thiochrome method.

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