Note

High-performance liquid chromatographic analysis of thiamine in rice flour with fluorimetric post-column derivatization

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(Received September 13th, 1983)

High-performance liquid chromatography (HPLC) has been widely applied in determining the nutrients in food products. HPLC determination of thiamine in multivitamin pharmaceutical preparations[1,2] and enriched cereal products[3] has been accomplished using an ultraviolet (UV) detector. The low sensitivity of the UV detector, however, is unsuitable for the analysis of foods that contain microgram amounts of thiamine. Recently, highly sensitive fluorimetric HPLC determinations of thiamine have been reported in which both pre-column derivatization (conversion of thiamine in meat products and selected foods to thiochrome before the chromatographic separation[4,5], and post-column derivatization (oxidation of thiamine in rats to thiochrome after the separation[6,7]) were used.

This paper describes the extraction of thiamine from rice flour according to the AOAC (1980) method[8] followed by HPLC determination with post-column derivatization.

EXPERIMENTAL

Apparatus

The system consists of an LC-3A pump for liquid chromatography, a SIL-1A injector, a Nucleosil 5 C_{18} column (15 cm × 4 mm I.D.; Macherey-Nagel, Düren, F.R.G.); CTO-2A column oven (55°C), a stainless-steel mixing coil (30 cm × 0.8 mm I.D.), a PRR-2A proportioning pump, an RF-530 spectrofluorimetric detector (excitation, 375 nm; emission, 435 nm) and a Chromatopac C-R1B recorder–integrator. All the instruments were purchased from Shimazu (Kyoto, Japan).

Reagents

Thiamine hydrochloride was obtained from Wako (Tokyo, Japan) and takadiastase from Sankyo (Tokyo, Japan). All other chemicals were of the best grade commercially available.

Sample preparation

Brown and polished rice of three cultivars (Sasanishiki, Koshihikari and To-yonishiki) and a commercial fortified rice were used. The polished rice was polished with a commercial polisher (Satake) to 90% and 93% of its initial weight to remove the husk and bran. All the rice investigated was ground in a laboratory mill to pass through a 30-mesh screen, then mixed by tumbling.

Thiamine extraction

The rice flour was decomposed by enzymatic hydrolysis with takadiastase according to the AOAC (1980) method[8], then centrifuged at 3500 rpm for 20 min. The supernatant was used as the sample.

HPLC procedure

Fig. 1 shows a schematic diagram of the HPLC method. A mixture of 0.01 M sodium dihydrogen phosphate and 0.15 M sodium perchlorate solution (adjusted to pH 2.2 with perchloric acid) was pumped at a flow-rate of 0.6 ml/min as the mobile phase. A 25 μl-volume of the supernatant described above was injected into the column, and 0.1% potassium hexacyanoferrate(III)–12% sodium hydroxide solution was provided at a flow-rate of 0.6 ml/min by a proportioning pump and mixed with the column eluate to convert thiamine to thiochrome. The thiochrome was measured with a spectrofluorimeter.

Determination of thiamine by the conventional method
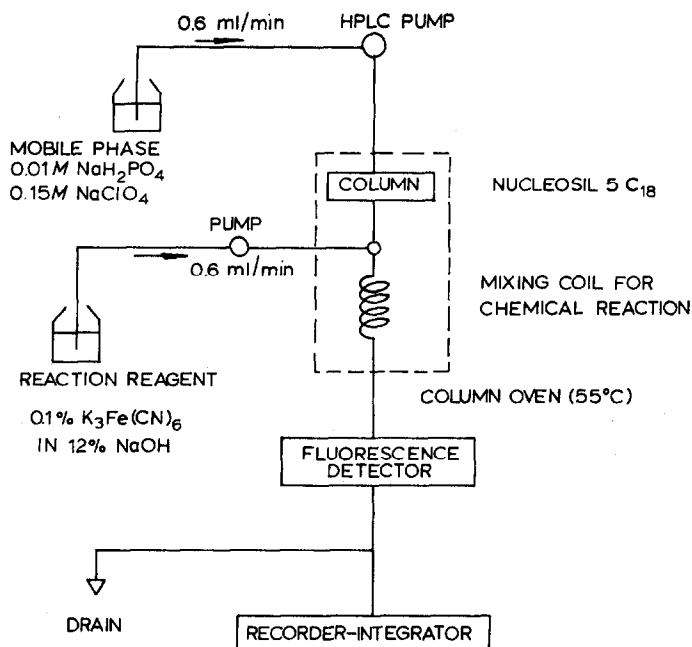
This was accomplished by the manual AOAC (1980) method[8].

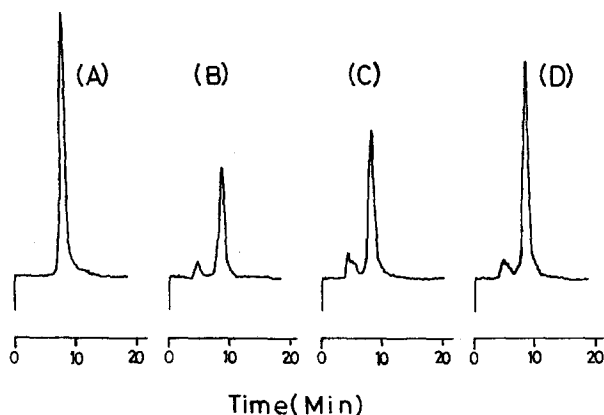Fig. 1. Schematic diagram of the HPLC system.

Fig. 2. Chromatograms of thiamine standard at 0.5 μl/ml (A), and thiamine extracts from 90% polished rice flour (B), 90% polished rice flour (C) and brown rice flour (D) of Sasanishiki cultivar. Sample size: 25 μl. For conditions, see text.

TABLE I

THIAMINE CONTENT OF RICE FLOUR DETERMINED BY THE HPLC AND THE AOAC METHODS

For the HPLC conditions, see text.

Cultivar	Sample	Thiamine (mg per 100 g)	
		HPLC*	AOAC**
Sasanishiki	Brown rice	0.46 ± 0.02	0.47
	Polished rice (93%)	0.32 ± 0.02	0.33
	Polished rice (90%)	0.19 ± 0.01	0.18
Koshihikari	Brown rice	0.51 ± 0.03	0.51
	Polished rice (93%)	0.35 ± 0.02	0.34
	Polished rice (90%)	0.23 ± 0.04	0.25
Toyonishiki	Brown rice	0.39 ± 0.03	0.36
	Polished rice (93%)	0.26 ± 0.01	0.28
	Polished rice (90%)	0.15 ± 0.02	0.15
	Fortified rice	1.07 ± 0.02	1.03

* Average ± standard deviation for four replicate analyses.
** Average of duplicate analyses.

RESULTS AND DISCUSSION

Fig. 2 shows the separation pattern of thiamine standard and thiamine extracts from rice flour (Sasanishiki cultivar). The fluorescent peaks were observed with a fluorescence detector at 4.5 and 8.5 min, respectively, indicating that the second peak corresponded to the thiamine standard. The thiamine peak, therefore, was free from interfering compounds.

Table I lists the thiamine contents in the rice flour determined by both the HPLC and the AOAC (1980) methods. Koshihikari rice flour had a greater thiamine concentration than other rice flour, and the thiamine concentration gradually decreased in the order brown rice flour, 93% polished rice flour and 90% polished rice flour because the bran had a high thiamine content.

The HPLC and AOAC methods gave comparable results; a paired t-test of simple average values showed no significant difference at the 5% level. A high correlation coefficient ($r = 0.998$) was obtained.

In order to determine the recovery of thiamine, we added the thiamine standard to brown rice flour (Sasanishiki cultivar) before the enzymatic hydrolysis. The amount of thiamine added to the rice flour was 0.2 mg per 100 g and the total recovered was 0.65 mg per 100 g, *i.e.*, a 95% recovery; several experiments gave recoveries in the range 92–96%.

The combination of HPLC and fluorescent detection has the advantage of sensitivity and selectivity in determining thiamine in rice flour. It is also simpler than the conventional technique as the thiochrome conversion step is eliminated and there is no need to extract thiamine with isobutanol, a suspected carcinogen[9]. Further, the analysis time was greatly reduced by the automatic sampler.

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