Determination of Thiamine and Its Esters in Beers and Raw Materials Used for Their Manufacture by Liquid Chromatography with Postcolumn Derivatization

PILAR VIÑAS, CARMEN LÓPEZ-ERROZ, NURIA BALSALOBRE, AND MANUEL HERNÁNDEZ-CÓRDOBA*

Department of Analytical Chemistry, Faculty of Chemistry, University of Murcia, E-30071 Murcia, Spain

Thiamine and its mono- and pyrophosphate esters were determined in beer and the raw materials used for its manufacture (brewer’s yeast, malt, raw grain, and hops) after separation using reversed-phase liquid chromatography. The method used fluorescence detection and a new amide-based stationary phase, which avoids the need to form ion pairs, leading to narrower peaks and a simpler mobile phase. Analyses were performed by isocratic elution with a phosphate buffer mobile phase and using a postcolumn derivatization system based on the oxidation of thiamine to fluorescent thiochrome with potassium ferricyanide in alkaline solution. Only thiamine was found in the beers and raw products, especially in brewer’s yeast and malt. A stability study pointed to a faster decrease in the thiamine content of samples stored at room temperature and in sunlight.

KEYWORDS: Liquid chromatography; fluorescence; thiamine; thiamine esters; thiochrome; beer

INTRODUCTION

Vitamins are complex organic substances that occur in the biological materials we consume as food. Thiamine (vitamin B₁) occurs in foodstuffs in its free form or as its mono- or pyrophosphate esters complexed with protein. Although it is extremely widespread in small amounts, only a few foodstuffs, generally those that are rich in carbohydrate, can be regarded as good sources. Examples are legume seeds and the germ of cereal grains, cereal products, vegetables, meat, and milk products (1). Thiamine is very labile in neutral to basic solutions but not in acidic solutions. Loss of thiamine may occur for mildly acidic to neutral of basic foods on heat treatment, but more acidic foods would not experience this loss. The greatest losses during domestic cooking as well as in commercial food processing occur when the vitamin is leached into the cooking water.

Beer is a natural beverage manufactured from malted barley, water, yeast, and hops. Vitamins are found among the nonvolatile components of beer, which contain all of the important vitamins of the B group. They come from malt, increase during barley germination, and remain during toasting. The mean concentration of thiamine in beer (2) has been established as 29 μg/L. The thiamine content of beer is important for nutritional information.

Thiamine cannot be distinguished from its esters by the usual analytical techniques such as the fluorimetric method proposed by the AOAC (3), and no distinction is made in tables of food composition. It is important to know the speciation of thiamine in beer because there are different activities for the thiamine esters and also different stabilities. Then, when it is necessary to distinguish between all of the naturally occurring species of thiamine, liquid chromatography (LC) is usually the method of choice (4). Procedures for the determination of thiamine in foods using reversed-phase (5–10) or ion pair chromatography (11–25) have been proposed, and several reviews have also been published (26–29). UV spectrophotometry (11, 13–15, 18, 21, 24, 25) has been used for samples that contain sufficient amounts of thiamine, while a fluorescence derivatization reaction based on the oxidation of thiamine to thiochrome, which shows strong fluorescence (5–7, 10, 12, 16–20, 23, 26, 28), is used for small concentrations. An alkaline solution of potassium hexacyanoferrate(III) is generally used as the oxidizing agent. However, no papers have been found concerning the determination of thiamine and its esters in beers and their raw materials.

In the present study, the separation of thiamine (T), thiamine monophosphate (TP), and thiamine pyrophosphate (TPP) is optimized by the reversed-phase technique without ion pair formation using a new amide-based stationary phase. Detection was performed by postcolumn fluorescence derivatization using a system involving the oxidation of the vitamins to the corresponding highly fluorescent thiochromes. The procedure could be applied to the determination of thiamine and its esters in beer and the raw materials used in its manufacture. Separation using the amide-based column is advantageous with respect to other existing methods (11–25) because the peaks are much narrower and column life is longer due to the simplicity of the mobile phase. Moreover, the use of the fluorescence detector...