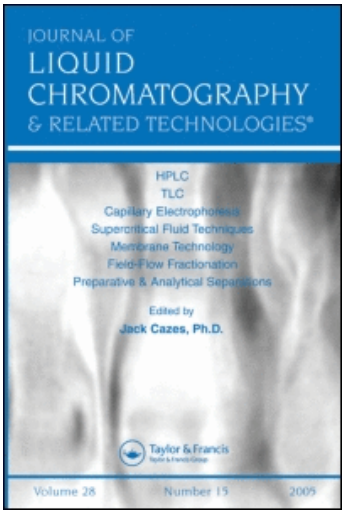

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

TLC Analysis of Corrinoid Compounds in Fish Sauce

Shigeo Takenaka[a]; Toshiki Enomoto[b]; Shingo Tsuyama[a]; Fumio Watanabe[c]
[a] Departments of Veterinary Science, Osaka Prefecture University, Sakai, Japan [b] Department of Food Science, Ishikawa Agricultural College, Ishikawa, Japan [c] Department of Health Science, Kochi Women's University, Kochi, Japan

Online publication date: 09 February 2003

To cite this Article Takenaka, Shigeo , Enomoto, Toshiki , Tsuyama, Shingo and Watanabe, Fumio(2003) 'TLC Analysis of Corrinoid Compounds in Fish Sauce', Journal of Liquid Chromatography & Related Technologies, 26: 16, 2703 — 2707
To link to this Article: DOI: 10.1081/JLC-120024540
URL: http://dx.doi.org/10.1081/JLC-120024540

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 26, No. 16, pp. 2703–2707, 2003

TLC Analysis of Corrinoid Compounds in Fish Sauce

Shigeo Takenaka,[1] Toshiki Enomoto,[2] Shingo Tsuyama,[1] and Fumio Watanabe[3,*]

[1]Departments of Veterinary Science, Osaka Prefecture University, Sakai, Japan
[2]Department of Food Science, Ishikawa Agricultural College, Ishikawa, Japan
[3]Department of Health Science, Kochi Women's University, Kochi, Japan

ABSTRACT

The amounts of vitamin B_{12} ($16.3 \pm 5.8\,\mu g/100\,g$) determined with the *Lactobacillus* microbiological method were about 5.4-fold greater in various fish sauces ($n = 15$) made in Japan than the values ($3.0 \pm 2.0\,\mu g/100\,g$) determined with the intrinsic factor-based chemiluminescence method. Corrinoid compounds found in the selected nine fish sauces were separated with silica gel 60 thin layer chromatography (TLC) and determined with the microbiological method, indicating that most B_{12}

*Correspondence: Fumio Watanabe, Department of Health Science, Kochi Women's University, Kochi 780-8515, Japan; E-mail: watanabe@cc.kochi-wu.ac.jp.

DOI: 10.1081/JLC-120024540
1082-6076 (Print); 1520-572X (Online)

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

is derived from unidentified corrinoid compounds. These results suggest that fish sauce may not be suitable for use as a vitamin B_{12} source.

Key Words: TLC; Bioavailability; Corrinoid; Fish sauce; Fermented foods; Vitamin B_{12}.

INTRODUCTION

Various kinds of fish sauces, traditional food supplements in the diet, are widely used in the world as condiments, and sometimes substituted for soy-bean sauces. A fish sauce (Nam-pla) appears to contribute a major source of vitamin B_{12} (B_{12}) in Thailand since it contains considerable amounts of B_{12}.[1,2] There is, however, little information available on whether B_{12} found in the fish sauce is true B_{12} or inactive corrinoid compounds for humans.

In the present paper, we describe thin layer chromatography (TLC) analysis of corrinoid compounds from various fish sauces.

EXPERIMENTAL

Materials

B_{12} was obtained from Wako Pure Chemical Industries (Osaka, Japan). A B_{12} assay medium for *Lactobacillus delbrueckii* subsp. *lactis* (formerly *L. leichmannii*) ATCC7830 was obtained from Nissui (Tokyo, Japan). Silica gel 60 TLC aluminium sheets were obtained from Merck (Darmstadt, Germany). Cyanocobamides (5-hydroxybenzimidazolyl cyanocobamide, benzimidazolyl cyanocobamide, and 7-adenylcyanocobaminde) isolated from bacteria were kindly provided by Dr. E. Stupperich, Ulm University, Germany. All other reagents used were of the highest purity available commercially. The fish sauces tested were obtained from a local market in Kanazawa-city, Ishikawa-prefecture, Japan, and purchased from a local market in Osaka-city, Osaka-prefecture, Japan.

A Shimadzu (Kyoto, Japan) UV-VIS spectrophotometer (UV-1600) was used to measure turbidity of *L. delbreuckii* test culture in the microbiological B_{12} assay method. A fully automated chemiluminescence B_{12} analyzer ACS 180 (Chiron Diagnostics, East Walpole, MA) was used for the B_{12} assay.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Methods

Extraction of B_{12} in Fish Sauces

Ten grams of each fish sauce were added to 10 mL of 0.1 mol/L acetate buffer, pH 4.8, containing 20 mg of KCN. Total B_{12} was extracted from the solution by boiling for 30 min at 98°C in the dark. The extraction procedures were done in a Dalton (Tokyo, Japan) draught chamber with fume hood. The boiled solution was cooled to room temperature and used for the B_{12} assay.

Assay of B_{12}

B_{12} was assayed by the microbiological method with *L. delbrueckii* ATCC 7830 and a B_{12} assay medium (Nissui, Tokyo, Japan), and by the fully automated chemiluminescence B_{12} analyzer ACS 180 as described previously.[3] The above B_{12} extracts were directly applied to the chemiluminescence B_{12} analyzer. They were diluted with distilled water up to a B_{12} concentration range of 0.01–0.2 μg/L and used as samples for the microbiological method. The turbidity (100-T%) of the *L. delbrueckii* test culture was measured at 660 nm with a Shimadzu spectrophotometer (UV-1600).

Thin Layer Chromatography Analysis

The B_{12} extracts of the selected nine fish sauces (containing >16 μg of B_{12}/100 g by determination with the microbiological method) were spotted on the silica gel 60 TLC sheet and developed with 1-butanol/2-propanol/water (10 : 7 : 10) as the solvent in the dark at 24°C. The TLC sheet was dried and cut into small pieces (0.5 × 1.0 cm) with scissors. B_{12} was extracted from the pieces in 80% (v/v) methanol containing 20 mg/L KCN several times, evaporated to dryness under reduced pressure, dissolved in 1.0 mL of distilled water, and used as samples for the B_{12} microbiological assay.

The concentrated solutions (2 μL) of authentic B_{12} and cyanocobamides were spotted on the silica gel 60 TLC sheet and developed under the same conditions. The TLC sheet was dried and R_f values of the pink-colored spots of the corrinoids were determined.

RESULTS AND DISCUSSION

Historically, B_{12} contents of foods have been determined by bioassay with B_{12}-requiring microorganisms; *L. delbrueckii* ATCC7830 has been used widely.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Recently, several workers have attempted to assay B_{12} in foods using a fully automated chemiluminescence B_{12} analyzer with the acridinium ester-labeled B_{12} derivative and hog intrinsic factor, the most specific B_{12}-binding protein, and has demonstrated that except for foods containing substantial amounts of inactive corrinoids, the observed correlation coefficient between the microbiological and chemiluminescence methods in foods is excellent.[3,4]

The amounts of B_{12} in various fish sauces ($n = 15$) made in Japan were determined with both *L. delbrueckii* microbiological and chemiluminescence methods. The values ($16.3 \pm 5.8\,\mu g/100\,g$) determined with the microbiological method were about 5.4-fold greater than the values ($3.0 \pm 2.0\,\mu g/100\,g$) determined with the chemiluminescence method.

B_{12} contents (0.3–$5.8\,\mu g/100\,g$) in the fish sauce made in Thailand (Nam-pla) have been determined by the radioisotope dilution assay method with radio-labeled B_{12} and the intrinsic factor.[2]

To evaluate why such differences between the values determined by both microbiological and chemiluminescence methods occur in the fish sauces,

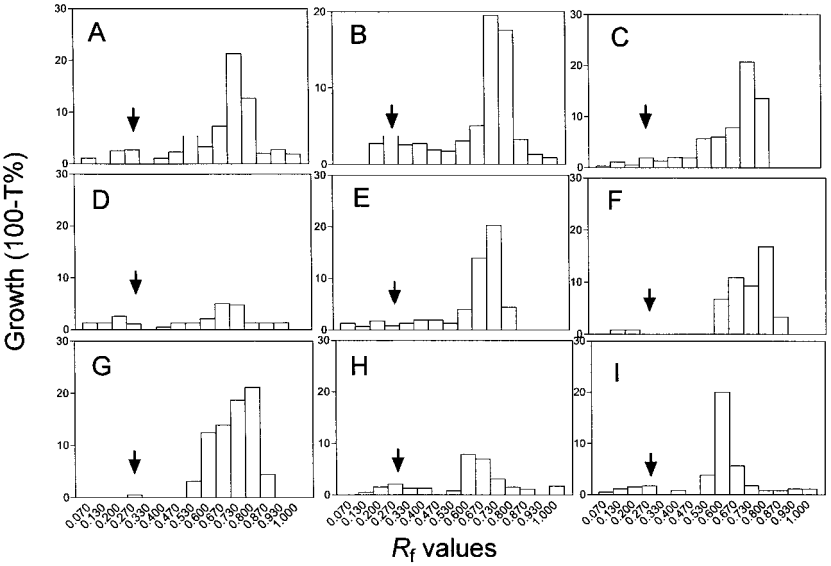

Figure 1. Mobile profiles of B_{12} active-compounds from the selected nine fish sauces (A ~ I) by silica gel 60 TLC. The TLC sheet developed with 1-butanol/2-propanol/water (10 : 7 : 10) as a solvent was dried and cut into small pieces. Corrinoid compounds were extracted, evaporated to dryness, and dissolved in 1.0 mL of distilled water. B_{12} was determined in these fractions with the microbiological assay. Arrows represent the fraction with R_f value of the authentic B_{12}. Data present typical mobile patterns of B_{12} active-compounds on the TLC from three experiments.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

corrinoid compounds found in the selected nine fish sauces (containing >16 μg of B_{12}/100 g by determination with the microbiological method) were separated with silica gel 60 TLC and determined with the microbiological method (Fig. 1). Although, most B_{12} determined by the microbiological method in each fish sauce was recovered in the fractions with R_f values 0.6–0.67 or 0.73–0.8 (or both), only several percentages of total B_{12} were found in the fraction with R_f value (0.23) of the authentic B_{12}. R_f values of the authentic naturally occurring corrinoid compounds, benzimidazolyl cyanocobamide, 5-hydroxybenzimidazolyl cyanocobamide, and 7-adenyl cyanocobamide, were 0.18, 0.20, and 0.14, respectively, in this TLC system.

Although, *L. delbrueckii* ATCC 7830 can utilize both deoxyribosides and deoxynucleotides (known as an alkali-resistant factor), as well as B_{12},[5] the alkali-resistant factor could not be detected in these fish sauces tested. These results suggest that the B_{12} active-fractions with R_f values of 0.6–0.67 and 0.73–0.8 are not derived from the alkali-resistant factor, but from unidentified corrinoid compounds.

Fish sauces may not be suitable for use as a B_{12} source because the unidentified corrinoid compounds are predominant in various fish sauces tested.

REFERENCES

1. Areekul, S.; Boonyananta, C.; Matrakul, D.; Chantachum, Y. Determination of vitamin B_{12} in fish sauce in Thailand. J. Med. Ass. Thailand **1972**, *55*, 243–248.
2. Areekul, S.; Thearawibul, R.; Matrakul, D. Vitamin B_{12} contents in fermented fish, fish sauce and soya-bean sauce. Southeast Asian J. Trop. Med. Pub. Hlth. **1974**, *5*, 461.
3. Watanabe, F.; Takenaka, S.; Abe, K.; Tamura, Y.; Nakano, Y. Comparison of a microbiological assay and a fully automated chemiluminescent system for the determination of vitamin B_{12} in food. J. Agric. Food Chem. **1998**, *46*, 1433–1436.
4. Yamada, K.; Yamada, Y.; Fukuda, M.; Yamada, S. Bioavailability of dried Asakusanori (*Porphyra tenera*) as a source of cobalamin (vitamin B_{12}). Int. J. Vitam. Nutr. Res. **1999**, *69*, 412–418.
5. Schneider, Z. Purification and estimation of vitamin B_{12}. In *Comprehensive B_{12}*; Scheider, Z., Stroinski, A., Eds.; Walter de Gruyter: Berlin, 1987; 111–155.

Received November 11, 2002
Accepted December 4, 2002
Manuscript 6108H

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016