Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 863 (2008) 88–93
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

High-performance liquid chromatography with fluorescence detection for quantitation of tryptophan and tyrosine in a shrimp waste protein concentrate

D.I. Sánchez-Machado, B. Chavira-Willys, J. López-Cervantes *

Departamento de Biotecnología y Ciencias Alimentarias, Instituto Tecnológico de Sonora, 5 de febrero 818 Sur, CP 85000, Ciudad Obregón, Sonora, Mexico

Received 11 September 2007; accepted 4 January 2008
Available online 16 January 2008

Abstract

A new, simple, and reproducible isocratic high-performance liquid chromatography (HPLC) method has been developed for the determination of free and total tyrosine and tryptophan in a protein concentrate. To determine total amino acids, the method involves alkaline hydrolysis of the proteins with sodium hydroxide at 120 °C for 4 h in the absence of air. Best results were achieved with a SS Exil ODS column 5 μm (25 cm × 0.46 cm i.d.), with an eluent of methanol: 40 mM sodium acetate buffer (adjusted to pH 4.5 with acetic acid; 20:80, v/v), a flow rate of 0.80 mL/min at 26 °C, and with programmable fluorescence detection. Under optimum conditions excellent linearity was obtained, and the overall recovery was 90.5, and 95.9% for total tryptophan and tyrosine, respectively. The precision results showed that the relative standard deviation of the repeatability and reproducibility were ≤4.78 and ≤4.65, respectively. This method was used to quantify the cited analytes in the protein concentrate obtained during the lactic acid fermentation of shrimp waste.

Keywords: Tryptophan; Tyrosine; High-performance liquid chromatography (HPLC); Protein concentrate; Shrimp waste; Fluorescence detection

1. Introduction

Amino acids are known to be precursors for a variety of biologically important substances, including many neuroactive compounds. The aromatic amino acids, tryptophan and tyrosine, are the most important in this respect [1–3]. In addition, tryptophan is an essential amino acid for many animals and for man, but its excessive dietary intake is reported to exert atherogenic effects [4,5]. This nutritional importance emphasizes the need for reliable analytical methods for the determination of tryptophan and tyrosine in food and feed proteins.

Free amino acids are usually analyzed without hydrolysis. However, the analysis of major amino acids requires a hydrolysis process (usually acid hydrolysis: 100–120 °C, 6 N HCl and 22–24 h). Due to its indole side chain, tryptophan is labile and poorly recovered in the presence of the acid and oxygen usually used in acid hydrolysis. Consequently, tryptophan has often been ignored in investigations concerning overall amino acid profile [6]. Numerous methods for determination of tryptophan in complex samples have been proposed. The majority of these procedures involve the following basic steps: (a) alkaline hydrolysis of samples at 100–125 °C in air-deprived medium for 16–20 h; (b) dilution of hydrolysate neutralized or not with cold concentrated HCl, with a chromatographic buffer; (c) clarification of dilute hydrolysate; (d) high-performance liquid chromatography (HPLC) separation; (e) spectrophotometric or fluorimetric determination [7]. The most effective alkalis used for protein hydrolysis are NaOH, $Ba(OH)_2$ and LiOH [8–11], with or without a carbohydrate or thiodiglycol as an antioxidant [12,13]. Several methods for tryptophan determination have been developed for pure protein and feed proteins involving ion-exchange chromatography or reversed-phase high-performance liquid chromatography with fluorometric or UV–visible detection after chemical derivatization [14–18]. Most underivatized amino acids (except those with aromatic side chains) do not possess suitable chromophores for spectroscopic detection except at very short wavelengths; the exception to that problem is the detection of tryptophan. Tryptophan and tyrosine exhibits rea-

* Corresponding author. Tel.: +52 644 4109000; fax: +52 644 4109001.
E-mail address: jlopezc@itson.mx (J. López-Cervantes).

1570-0232/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2008.01.011

sonably strong UV absorbance at longer wavelengths [19,20]. Moreover, tryptophan exhibits a strong native fluorescence that has been employed for facile detection of free tryptophan [21,22].

Shrimp is the major seafood captured in Sonora, México. As shrimp is processed for exportation, a considerable volume of proteinaceous by-products including shrimp heads and shells are generated. This waste is rich in protein and chitin [23–26], and can be transformed to a silage by the process of lactic fermentation [27,28]. The fermented product obtained contains a protein-rich liquor, and an insoluble fairly clean chitin [29]. Due to its high protein and mineral content this fermented product has been incorporated into animal feeds [30]. The studies of the protein isolated from seafood waste have focused on the amino acid composition of the protein removed from crawfish shell [31], shrimp shell [32], protein hydrolysate from demineralized shells prepared using enzymes [33], and fermented shrimp waste [34], some which has been reported to be rich in essential amino acids. Little is known about the tryptophan content in the protein concentrate from shrimp waste [35], whether if there is information available of the tyrosine contents in protein from that waste.

In the present work, a selective and sensitive method for the determination of tryptophan and tyrosine was developed using alkaline hydrolysis and a liquid chromatographic system with fluorescent detection. The method was optimized and evaluated for protein concentrate from fermented shrimp waste, and the use of the method was demonstrated.

2. Experimental

2.1. Chemicals and standards

HPLC grade methanol was obtained from EMD Chemicals (Darmstadt, Germany). Tyrosine and tryptophan standards were purchased from Sigma (St. Louis, MO, USA; Fig. 1). The purity of the reference standards were ≥99.0%. Analytical grade sodium acetate, sodium hydroxide, boric acid and hydrochloric acid were obtained from Productos Monterrey (Monterrey, Nuevo Leon, México). All aqueous solutions were prepared with ultra-pure water purified with the NANOpure Diamond UV system (Barnstead International, Dubuque, Iowa, USA). The borate buffer was prepared from 250 mM boric acid solution adjusted to pH 8.5 with 1 M sodium hydroxide solution prepared from sodium pellets.

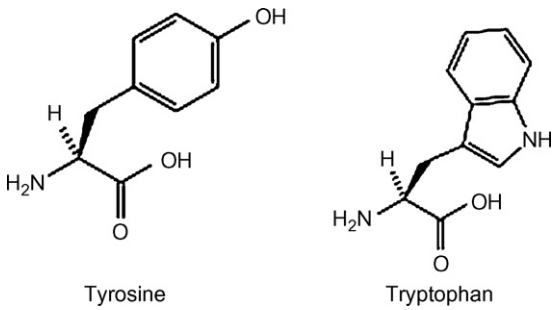

Fig. 1. Chemical structure of tryptophan and tyrosine.

Standard stock solutions of tyrosine (326 μg/mL) and tryptophan (404 μg/mL) were prepared in acidic water (ultra-pure water adjusted to pH 6.3 with 0.1 M hydrochloric acid) and stored at 4 °C away from light. Working solutions for tyrosine and for tryptophan were prepared from those solutions and diluted with acidic water, and then seven concentration levels were analyzed. The stock solutions were prepared every 2 weeks, and the working solutions were prepared the same day that they were used. An aliquot (5 μL) of each standard working solution was subject to HPLC analysis. For quantification, peak areas were correlated with the concentrations according to the calibration curve.

2.2. Instrumentation and chromatographic conditions

The HPLC system (GBC, Dandenong, Australia) was equipped with an auto injector LC1650, an on-line solvent degasser LC1460, a system controller WinChrom, a pump LC1150, a column oven LC1150, a 5 μL injection loop (Rheodyne, Cotati, CA, USA), and a fluorescence detector LC1255S.

The chromatographic analysis was performed using an analytical scale (25 cm × 0.46 cm i.d.) SS Exil ODS column with a particle size 5 μm (SGE, Dandenong, Australia). The HPLC conditions were as follows: the mobile phase consisted of the mixture of methanol:40 mM sodium acetate buffer (adjusted to pH 4.5 with acetic acid; 20:80, v/v) filtered through a 0.22 mm membrane and degassed; a flow rate of 0.80 mL/min; column temperature was 26 °C. The fluorescence was recorded at the optimal wavelength for tyrosine (λ_{ex} = 274 nm and λ_{em} = 304 nm) for 5.8 min, followed by the optimal wavelength for tryptophan (λ_{ex} = 280 nm and λ_{em} = 348 nm) for another 9.2 min. These conditions were based on preliminary trials from method described by Landry and Delhaye [7] for isocratic liquid chromatography, with minor modifications.

2.3. Samples

Slightly thawed minced shrimp waste samples were fermented at 30 °C for 36 h. The silage was centrifuged to obtain the chitin-rich fraction (sediment or raw chitin), the protein-rich liquor, and the lipid fraction [34]. The protein-rich liquor was freeze-dried or dried and ground. After that, the samples were conserved in desiccators in darkness until their analysis.

2.4. Extraction of free tryptophan and tyrosine

To evaluate free tryptophan and tyrosine, amino acids were extracted from the freeze-dried or dried samples with acidic water. Specifically, a 25 mg finely ground sample was placed in a volumetric flask and diluted to 50 mL with acidic water to obtain a concentration of 0.50 mg/mL. Afterwards, the samples were sonicated for 2 min for completed dissolution.

2.5. Hydrolysis of proteins

Samples (25 mg) were weighed in Pyrex glass tubes with Teflon-lined screw-caps used as the hydrolysis vessel. Three

millilitres of 4.2 M sodium hydroxide were then added to the glass tubes and the content was mixed in an ultrasonic bath for 2 min. Air was removed with nitrogen. Subsequently, the samples were hydrolysed by heating at 120 °C for 4 h and cooled in an ice bath. Then, the pH was adjusted to 9 with concentrated hydrochloric acid. The resulting solution was vacuum-filtered through Whatman no. 41 paper. The filtrate was diluted to 50 mL with a borate buffer in a volumetric flask. Then, 5 μL of the final solution was injected into HPLC.

2.6. Statistical analysis

For statistical analyses, the computer program was SPSS 11.0 for Windows (SPSS Inc., Chicago, IL). The relative standard deviation (RSD, %) is the ration standard deviation-average expressed as a percentage.

3. Results and discussion

The analysis of tryptophan has been historically problematic, due to the fact that alkaline hydrolysis steps are time-consuming and require hazardous reagents for hot hydrolysis. In view of the problem observed by researchers, most of the HPLC methods for tryptophan determination are based on the same principle, with modification only in the hydrolysis time, temperature and concentration of NaOH [5,6,10–12]. The purpose of this work was to develop a specific and accurate HPLC method for tryptophan and tyrosine that could be routinely applied to the quantitation of these compounds. To keep the procedure as simple and reliable as is possible, all analytical steps were thoroughly investigated. Now, we present a method that has some advantages by employing the native fluorescence of both amino acids and a short alkaline hydrolysis time. With this characteristic, the procedure has an accuracy and reproducibility that are acceptable.

3.1. HPLC separation and identification

To determine optimal chromatographic conditions for the determination of tryptophan and tyrosine, the lab performed preliminary trials with standards. In the direction of the mobile phase, Landry and Delhaye [7] have recommended using methanol and sodium acetate buffer (20:80), the trials were performed with sodium acetate buffer at 40, 70 and 100 mM (pH 4.5 and 5.0) and four mobile phase flow rates (0.6, 0.8, 1.0 and 1.2 mL/min), but without varying the phase compositions. The best peak resolution was obtained with methanol:40 mM sodium acetate pH 4.5 (20:80) and 0.8 mL/min. These conditions were then used in trials of three different column temperatures (26, 30, and 34 °C), in which the best results were obtained at 26 °C.

The tryptophan and tyrosine were identified by comparison of retention times against those obtained from amino acids stock solutions. The fluorescence excitation and emission wavelengths used for both amino acids are recommended because the peak showed the major intensities. Chromatograms of the mixture of tyrosine and tryptophan standards, and of a prepared sample of protein concentrate from fermented shrimp waste, both obtained under the optimized conditions, are shown in Fig. 2A and B, respectively. Peaks were observed at 4.25 ± 0.01 min for tyrosine, and 8.32 ± 0.02 min for tryptophan with an average of 12 injections, and fell in the range of typical chromatographic separation.

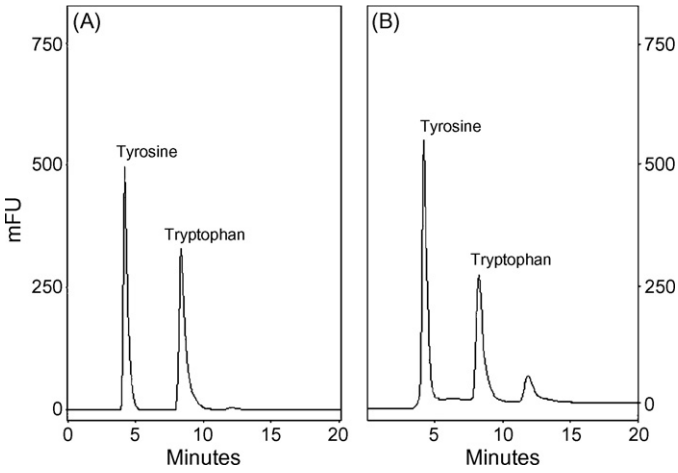

Fig. 2. HPLC chromatograms of standard solution of tryptophan and tyrosine (A) and a sample of protein concentrate from fermented shrimp waste (B).

3.2. Free tyrosine and tryptophan extraction

For the extraction of free tryptophan and tyrosine, the sample preparation method was simple and consisted of diluting the freeze-dried or dried protein concentrate in the selected solvent (acidic water). For the determination of the amount of sample used in the chromatographic analysis, assays were made in different concentrations from sample powder (1, 2 and 3 mg/mL) with different time lengths of sonification (0, 2 and 4 min), all of them dissolved in acidic water. Optimal conditions were identified on the basis of peak areas in chromatography.

3.3. Optimum conditions of alkaline hydrolysis of protein

For many years, numerous researchers have considered tryptophan more stable to alkali than acid, so that basic hydrolysis of proteins would be preferable for tryptophan analysis. However, the methods of alkaline hydrolysis are either time-consuming or inaccurate [4]. In recent times, starch, lactose and ascorbic acid have been used as antioxidants to protect tryptophan against oxidation in the alkaline hydrolysis of proteins and feed stuffs. These additives remove residual traces of oxygen in the hydrolysis by incorporating the oxygen into products that do not react with tryptophan [10]. During alkaline hydrolysis of protein, the concentration of NaOH, temperature and hydrolysis time are prime attributes affecting tryptophan recovery [9]. To optimize these conditions, the following experiments were carried out. The volume of NaOH (3, 6 and 9 mL), and hydrolysis time (4, 8, 12 and 16 h) were the optimized variables with the constant sample amount (25 mg). Temperature and concentration of the NaOH (4.2 N) have been recommended in early studies [22]. In all trials, the air was removed with nitrogen and two samples were processed, setting the conditions. The results of the hydrolysis of the samples are presented in Fig. 3. When hydrol-

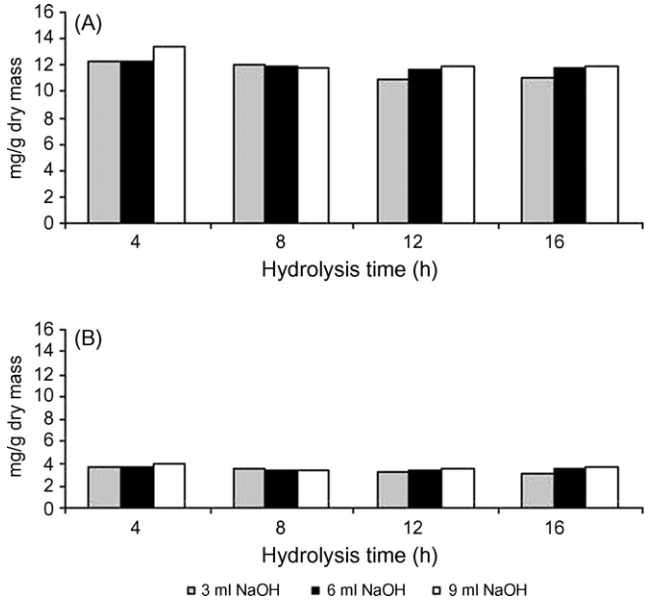

Fig. 3. Effect of hydrolysis time and volume of NaOH on the average ratio of total tryptophan and tyrosine. (A) Tyrosine; (B) Tryptophan.

ysis time was 4 h, for both amino acids, the recovery increased as the volume of NaOH increased from 3 to 9 mL. These results indicated that 4 h of hydrolysis time at 9 mL of 4.2 N NaOH is adequate for complete hydrolysis of proteins, but this amount of alkali increased the amount of acid for its neutralization. With 3 mL volume NaOH and 12 or 16 h hydrolysis time, the recovery was very low for tryptophan and tyrosine. It may seem that the hydrolysis time is the most important variable influencing the average ratio. Contrary to the finding in a recent report [5], these results unequivocally show that a short hydrolysis time proceeds without loss of tryptophan or tyrosine, as noted in Section 3.4. Due to these facts, 4 h of hydrolysis time and 3 mL 4.2 N NaOH were chosen for further works.

3.4. Method validation

Linearity was checked for each amino acid using six standard solutions with concentrations ranging in concordance with the level of the compound found in a protein concentrate from fermented shrimp waste. Regression equations for the calibration plots for tyrosine and tryptophan are showed in Table 1. The relationship between concentrations and peak area were always linear, with coefficients of determination greater than 0.999.

The precision study was comprised of repeatability and reproducibility studies. For free tryptophan and tyrosine, a total of 10 procedures were performed in replicate on a sample under optimum conditions to determine repeatability, and three replicate analyses of the same sample were made on different days to determine reproducibility, Table 2 shows these precision results. Additionally, the repeatability, for the total of the tyrosine and tryptophan, was researched by analyzing the same sample, and the RSD was found to be 2.16% (3.83 μg/g dry mass, $n = 8$) for tryptophan and 1.86% (12.96 μg/g dry mass, $n = 8$) for tyrosine. The overall relative standard deviations (RSDs) of the repeatability and the reproducibility for both amino acids are acceptable [11], which is sufficient for routine analyses. These results indicate that the present method can be used for quantitative analyses of these amino acids in a protein concentrate from fermented shrimp waste.

Table 1
Calibration curves of tryptophan and tyrosine

Compound	Range (μg/mL)	Equation	r^2
Tryptophan	0.48–3.39	$y = 5901.61x - 157.84$	0.9998
Tyrosine	1.17–8.22	$y = 2350.87x - 33.34$	0.9998

x, amount (μg/mL); y, peak area; r^2, determination coefficient.

Table 2
Precision of the method for determination of free tryptophan and tyrosine

Compound	Repeatability ($n = 10$)		Reproducibility ($n = 3$)	
	Mean ± SD[a]	RSD (%)	Mean ± SD[a]	RSD (%)
Tryptophan	2.91 ± 0.14	4.78	2.84 ± 0.13	4.65
Tyrosine	10.46 ± 0.38	3.63	10.38 ± 0.21	2.02

[a] Results expressed as mg/g dry mass.

Determination of detection limits for tryptophan and tyrosine (three times the basis of signal-to-noise ratio, as per American Chemical Society guidelines [36]) were 2 and 4 pg/mL, respectively.

Accuracy was estimated by means of recovery assays. For evaluation of recovery in free tryptophan and tyrosine, three spiking levels, used as assays, were triplicated, prior to extraction, and quantitation. Table 3 presents the recovery percentages obtained to free tryptophan and tyrosine. During the recovery for total tryptophan and tyrosine, eight concentrated powdered protein samples from fermented shrimp waste were spiked with a known concentration (1.56 mg/g dry mass and 3.85 mg/g dry mass) before hydrolysis, extraction, and quantitation. The recovery for total tryptophan and tyrosine was 90.50% (7.38% RSD) and 95.9% (2.33% RSD), respectively. These values are similar to those reported in previous studies [11].

3.5. Tryptophan and tyrosine (free and total) contents in a protein concentrate from fermented shrimp waste

The tryptophan and tyrosine contents of the freeze-dried and dried protein concentrate were determined in separate preparations and analysis of eight different batches of each. In all cases, the tyrosine content was higher than the tryptophan content (Table 4). The mean content of total tyrosine (10.78 ± 2.00 mg/g dry mass) was about three times that in total tryptophan (3.65 ± 0.46 mg/g dry mass) in freeze-dried samples. This pattern was also shown for dried samples. These differences may be attributable to the fact that the tryptophan is a limiting factor in protein [37] or that the tryptophan content can be reduced in the protein hydrolysates [38]. Furthermore, the free tyrosine and tryptophan mean contents were, respectively, approximately 67% and 74% of their values in the total tyrosine and tryptophan for both protein concentrates. Because of that, during fermentation, the shrimp waste is deproteinized by proteolytic enzymes produced by the added microorganisms

Table 3
Accuracy of the assay for free tryptophan and tyrosine

	Original (mg)	Spiked (mg)	Determined (mg)	Recovery (%)	Average (%)	RSD (%)
Tryptophan	3.09	0.63	3.67	92.06	95.88	3.64
	–	1.21	4.26	96.69	–	–
	–	1.82	4.89	98.90	–	–
Tyrosine	11.07	1.52	12.45	90.79	96.86	5.42
	–	2.92	13.99	99.91	–	–
	–	4.47	15.53	99.87	–	–

Table 4
Free and total tryptophan and tyrosine contents in protein concentrate from shrimp waste

Sample (batch number)	Tryptophan (mg/g dry mass)		Tyrosine (mg/g dry mass)	
	Free	Total	Free	Total
Freeze-dried				
1	2.08	3.59	6.04	9.69
2	2.48	3.50	5.72	9.52
3	2.62	3.43	6.29	9.80
4	2.65	3.45	6.60	9.88
5	2.91	4.11	4.91	8.10
6	3.03	4.57	8.75	12.90
7	2.53	3.40	9.35	12.79
8	3.01	3.13	10.53	13.57
Dried				
1	2.52	2.82	4.72	7.43
2	1.69	2.87	6.31	12.03
3	2.04	2.78	8.93	11.17
4	2.47	2.95	7.96	10.37
5	2.48	3.46	6.52	9.91
6	2.70	3.68	7.47	11.11
7	2.14	2.79	7.79	11.21
8	2.61	3.19	2.37	5.70

[39] and by the presence of proteolytic enzymes in the viscera [28]. In relation to this, tryptophan has been reported as 6.3 mg/g of dry mass for fermented shrimp heads stored for 180 days measured colorimetrically [27]. Meanwhile, free tyrosine was 56.9 mg/g of dry mass in freeze-dried protein fraction from shrimp waste hydrolysate [34]. Finally, it should be pointed out that free tyrosine and tryptophan contents of these concentrated samples, doubtlessly, depend on the fermentation procedure and not of the dehydrated method. This is achieved during the enzymatic deproteinization of the shrimp waste by the action of the microorganisms [40].

4. Conclusions

The HPLC method presented in this study is simple, rapid, accurate and reliable for determination of tryptophan and tyrosine (free and total) contents in protein concentrate from fermented shrimp waste. The method requires only a small sample volume and needs minimal manual sample preparation. For this reason, the method could be useful for quality control of both amino acids in food and feed supplements. This method could also be applied to biochemical and nutritional research.

Acknowledgements

This research was financed under project no. SON-2004-C03-016 with Mixed Funds of the Government of the State of Sonora and the National Council for Science and Technology (FOMIX-CONACYT). Michael A. Bryan, MBA and Native English Professor, offered suggestions and improvements in the wording of this manuscript.

References

[1] A. Ravikumar, K.V. Deepadevi, P. Arun, V. Manojkumar, P.A. Kurup, Neurol. India 48 (2000) 231.
[2] A. Vaarmann, A. Kask, U. Mäeorg, J. Chromatogr. B 769 (2002) 145.
[3] A. Amirkhani, E. Heldin, K.E. markides, J. Bergquist, J. Chromatogr. B 780 (2002) 381.
[4] M. Friedman, J.W. Finley, J. Agric. Food Chem. 19 (1971) 627.
[5] G. Ravindran, W.L. Bryden, Food Chem. 89 (2005) 309.
[6] F.C. Wu, E. Tanoue, Chem. Geol. 184 (2002) 139.
[7] J. Landry, S. Delhaye, J. Agric. Food Chem. 40 (1992) 776.
[8] J. Landry, S. Delhaye, G. Viroben, J. Agric. Food Chem. 36 (1988) 51.
[9] S. Delhaye, J. Landry, J. Agric. Food Chem. 41 (1993) 1633.
[10] F. Wu, E. Tanoue, Anal. Sci. 17 (2001) 1063.
[11] C. Delgado-Andrade, J.A. Rufián Henares, S. Jiménez-Pérez, F.J. Morales, Food Chem. 98 (2006) 580.
[12] T.E. Hugli, S. Moore, J. Biol. Chem. 247 (1972) 2828.
[13] A. Alegría, R. Barberá, R. Farré, M. Ferrerés, M.J. Lagarda, J.C. López, J. Chromatogr. A 721 (1996) 83.
[14] J. Vignau, M.-C. Jacquemont, A. Lefort, M. Imbenotte, M. Lhermitte, Biomed. Chromatogr. 18 (2004) 872.
[15] I. Molnár-Perl, M. Pintér-Szakács, M. Khalifa, J. Chromatogr. 632 (1993) 57.
[16] Y. dale, V. Mackey, R. Mushi, A. Nyanda, M. Maleque, J. Ike, J. Chromatogr. B 788 (2003) 1.
[17] J.L. Bernal, M.J. Nozal, J.C. Diego, A. Ruiz, J. Sep. Sci. 28 (2005) 1039.
[18] M. Yang, S.S. Tomelli, Anal. Chim. Acta 209 (2000) 45.
[19] M.M. Yust, J. Pedroche, J. Girón-Calle, J. Vioque, F. Millán, M. Alaiz, Food Chem. 85 (2004) 317.
[20] A.T. Wood, M.R. Hall, J. Chromatogr. B 744 (2000) 221.
[21] Y. Chen, M.D. Barkley, Biochemistry 37 (1998) 9976.
[22] T.J. Kivi, in: L. Nollet (Ed.), Food Analysis b HPLC, Marcel Dekker, New York, 2000, p. 321.
[23] Z. Zakaria, G.M. Hall, G. Shama, Process Biochem. 33 (1998) 1.
[24] L.A. Cira, S. Huerta, G.M. Hall, K. Shirai, Process Biochem. 37 (2002) 1359.
[25] P. Li, X. Wang, R.W. Hardy, D.M. Gatlin, Aquaculture 236 (2004) 485.
[26] A.M. Balogun, Y. Akegbejo-Samsons, Bioresour. Technol. 40 (1992) 157.
[27] O.A. Fagbenro, Food Rest. Int. 29 (1996) 595.
[28] M.S. Rao, W.F. Stevens, Food Technol. Biotechnol. 44 (2006) 83.
[29] I. Guerrero Legarreta, Z. Zakaria, G.M. Hall, in: A. Domard, C. Jeuniaux, R. Muzzarelli, G. Roberts (Eds.), Advances in Chitin Science, Jacques André, Francia, 1996, p. 399.

[30] D.J. Evers, D.J. Carroll, Anim. Feed Sci. Technol. 59 (1996) 233.
[31] H.K. No, S.P. Meyers, K.S. Lee, J. Agric. Food Chem. 37 (1989) 575.
[32] S.O. Andersen, Comp. Biochem. Physiol. 99B (1991) 453.
[33] J. Synowiecki, N.A.A.Q. Al-Khateeb, Food Chem. 68 (2000) 147.
[34] J. López-Cervantes, D.I. Sánchez-Machado, J.A. Rosas-Rodríguez, J. Chromatogr. A 1105 (2006) 106.
[35] O.A. Fagbenro, O.A. Bello-Olusoji, Food Chem. 60 (1997) 489.
[36] American Chemical Society (ACS), Subcommittee of Environmental Analytical Chemistry, Anal. Chem. 52 (1980) 2242.
[37] S. Diem, J. Bergmann, M. Herderich, J. Agric. Food Chem. 48 (2000) 4913.
[38] F. Shahidi, X.-Q. Han, J. Synowiecki, Food Chem. 53 (1995) 285.
[39] M.S. Rao, J. Muñoz, W.F. Stevens, Appl. Microbiol. Biotechnol. 54 (2000) 808.
[40] J. You, Y. Zhang, Chomatographia 56 (2002) 43.