ELSEVIER

Journal of Chromatography A, 815 (1998) 272–277

JOURNAL OF CHROMATOGRAPHY A

Short communication

Gas chromatography determination of polychlorinated biphenyls in powdered and liquid soybean milks

L. Ramos[a,*], M. Torre[b], M.L. Marina[a,b]

[a]*Centro de Tecnología de los Alimentos y Servicios Biosanitarios, Universidad de Alcalá, 28871 Alcalá de Henares, Madrid, Spain*
[b]*Departamento de Química Analítica, Facultad de Ciencias, Universidad de Alcalá, 28871 Alcalá de Henares, Madrid, Spain*

Received 15 January 1998; received in revised form 4 June 1998; accepted 8 June 1998

Abstract

A method previously validated for the analysis of individual congeners of polychlorinated biphenyls (PCBs) in soybean infant formulas is now used to determine PCB levels in dairy-like soybean derivatives, including liquid and powdered soybean milks. Some differences were found among PCB levels and profiles calculated from the different liquid and powdered soybean milks investigated when compared to previously reported for soybean infant formulas, and those most common in dairy-products from Spain and from other countries. PCB levels detected in liquid soybean milks were lower than those found in powdered soybean milks. Levels reported for these two dairy-like derivatives were lower than those detected in soybean infant formulas. The most apparent difference among soybean derivatives and cow's milk was that individual PCB levels detected in the former were lower than those reported for the latter, except for congeners 126 and, specifically, 77, which showed the opposite trend. This feature was common to all soybean products investigated including infant formulas, powdered and liquid soybean milks.

Keywords: Milk, soybean; Soybean; Food analysis; Polychlorinated biphenyls

1. Introduction

Polychlorinated biphenyls (PCBs) are a group of pollutants widespread in the environment due to their ample use in the past. Among the 209 possible isomers, attention is usually focused on those congeners that are stereoisomers of the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, which show a toxicity similar to that of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) [1]. In fact, determination of toxic coplanar PCBs has been proposed as a useful tool for evaluating the potential toxic health risk of samples [2–4]. From this point of view, a significant number of papers which have shown that the presence of these pollutants in fatty foods can be of special interest due to their direct incidence on humans [3,5,6]. This is cause for concern especially in the case of dairy products because of their widespread consumption by the population at large [7].

Owing to the nutritional properties of soybean, soybean-based dairy-like derivatives are proposed as one of the most interesting alternatives for people allergic to animal whey proteins. Due to the increase in the consumption of these types of products, an effort has been made in recent years to ascertain their

*Corresponding author. Present address: Faculteit der Scheikunde, Analytische Chemie, Vrije Universiteit, de Boelelaan 1083, 1081 HV Amsterdam, Netherlands.

0021-9673/98/$19.00
PII: S0021-9673(98)00504-4

nutritional characteristics [8]. Nevertheless, so far, the information reported concerning organohalogenated pollutant levels such as PCBs in these types of dairy-like products is scarce [5,9].

In this study, an analytical method previously validated for individual PCB analysis in soybean infant formulas [9], was applied to the PCB analysis in different dairy-like derivatives, including eight different brands of liquid soybean milk and four different samples of powdered soybean milk (three brands). In all cases, the toxic tetraequivalents (TEQs) of tetrachlorinated dibenzo-*p*-dioxin (2,3,7,8-TCDD) due to these pollutants were calculated. These levels were compared to those found in some dairy products from Spain and from other countries.

2. Experimental

2.1. Chemicals

All solvents were pestipur quality and were purchased from SDS (France). Anhydrous sodium sulphate (Panreac, Spain), Silica gel 60 (Merck, Germany) and Florisil (Sigma, USA) were used as adsorbents.

The 15 PCB congeners studied were selected based on their toxicity and relative abundance in biological and environmental samples according to the World Health Organisation (WHO) criteria [1]. Individual isomers were purchased from Ehrenstorfer (Germany). A working stock solution was prepared from individual coplanar PCB standards (PCBs IUPAC Nos. 28, 77, 101, 105, 118, 126, 138, 151, 153, 156, 167, 169, 170, 180 and 194) [10] containing 0.77 ng/μl of each in hexane. Two individual PCBs (No. 12, 3,4-dichlorobiphenyl and No. 209, 2,2′,3,3′,4,4′,5,5′,6,6′-decachlorobiphenyl) were used as external standards in the instrumental analysis step by high-resolution gas chromatography equipped with electron-capture detection (HRGC–ECD).

The eight different liquid soybean milks and the four different powdered soybean milks analysed were selected among the best well-known brands in Spain. They were purchased from supermarkets in Madrid (Spain).

2.2. Instrumentation

PCB sample extracts were analysed by HRGC–ECD (HP 5890 Series II) using helium and nitrogen as carrier gas and auxiliary gas, respectively. Confirmation of the individual PCB congeners was developed by HRGC–low-resolution mass spectrometry (LRMS) using a HP 5890 Series II gas chromatograph coupled to a HP 5971 A mass spectrometer in the selected ion monitoring (SIM) mode. Helium was used as the carrier gas.

The fused-silica capillary BPX.5 column (60 m× 0.25 mm I.D., 0.25 μm film thickness) was purchased from SGE (Australia). Samples were injected in the splitless mode (0.60 min).

2.3. Procedure

A previously validated method for the analysis of PCBs in soybean infant formulas [9] was applied in this work. Briefly, a 20-g sample was mixed with 5 g of silica gel and 20 g of anhydrous sodium sulphate, loaded into a column, and extracted with 300 ml of an acetone–hexane (1:1, v/v) mixture. No special sample preparation was required, except for liquid samples that were previously freeze-dried. Total lipids extracted were determined gravimetrically after eliminating solvents. Subsequently, a clean-up multi-step of the fatty extracts using SiO_2–H_2SO_4 [11] and Florisil [12] columns was carried out. A blank sample was run every four samples to check any contamination throughout the analytical procedure.

The residue containing the PCBs was taken up in a working solution containing PCBs 12 and 209. These individual congeners were added just before the chromatographic injection in order to correct injection errors and detector fluctuations.

The column temperature program used was 60°C (1 min) to 190°C (1 min) at 60°C/min, then to 243°C (45 min) at 3.3°C/min, and then to 268°C (45 min) at 1.9°C/min. The injector and detector temperatures were 280°C.

Identification of individual PCB congeners by HRGC–ECD was based on the comparison of their retention times related to PCB 209 with those of the stock solution mixture. Quantification of PCB levels was based on individual peak areas and the response

factors of the individual congeners related to PCB 209. Confirmation of the individual PCB isomers by HRGC–LRMS was based on the simultaneous detection, at the appropriated retention time, of the chromatographic signals corresponding to the two masses selected for each congener, and on the maintenance of their ratios within the range (±10%) of the previously calculated theoretical ratio [13].

3. Results and discussion

The precision and the accuracy of the proposed analytical procedure were presented in a previous paper [9]. Mean recoveries for the spiked PCB congeners studied were in the 88–114% range, with relative standard deviations (R.S.D.s) lower than 9.8%. The R.S.D.s related to the determination of endogenous PCBs were in the 1.5–10.0% range. In that study, a detailed discussion about the ability of the BPX.5 chromatographic column to allow some critical separations of the coplanar CBs from their most common interfering compounds in 5% phenylpolysiloxane type columns [14] was included. The analysis of complex mixtures of PCBs (Arochlor 1260 and spiked Arochlor 1260) by HRGC–LRMS showed that, under the experimental chromatographic conditions finally proposed (see Section 2.3), a good resolution among the toxic PCBs 77 and 126 and their interfering congeners (PCB 110 and PCBs 129 and 178, respectively) was achieved. Furthermore, a good chromatographic separation of other PCB pairs such as 118–149 and 153–105 was achieved. No additional coelution problems were detected during the HRGC–LRMS analysis of the toxic congeners investigated in such a complex polychlorinated biphenyls mixtures.

3.1. PCB analysis in liquid soybean milks

PCB levels in eight different liquid soybean milk brands available in Spain were investigated (Table 1). PCBs 28, 77, 101, 118, 126, 138, 153, 167, 170 and 180 were found at quantifiable levels in all samples analysed, whereas congeners 169 and 194 were below their limits of detection. PCBs 105, 151 and 156 were found at quantifiable levels only in some of the investigated brands.

Among the individual isomers studied, the most abundant were PCBs 101, 77, 153, 28 and 138, with 0.78, 0.62, 0.46, 0.37 and 0.25 ng/g of lipids as mean concentrations, respectively. These congeners were also the most abundant in the different soybean infant formulas previously analysed [9]. However,

Table 1
Results of the analysis of eight different soybean liquid milks (ng/g of lipids)

PCB	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Mean	S.D.
28	0.88	0.59	0.27	0.51	0.22	0.10	0.11	0.24	0.37	0.27
101	1.15	1.46	1.25	0.46	0.32	0.40	0.27	0.91	0.78	0.47
77	0.76	1.04	0.53	1.14	0.49	0.38	0.12	0.51	0.62	0.34
151	0.23	<0.02	0.06	<0.03	<0.02	0.06	0.02	0.11	0.06	0.08
118	0.12	0.41	0.15	0.12	0.10	0.11	0.06	0.15	0.15	0.11
153	0.42	0.80	0.55	0.48	0.28	0.50	0.15	0.46	0.46	0.19
105	0.72	0.14	0.05	0.02	<0.01	0.13	0.01	<0.01	0.13	0.24
138	0.24	0.46	0.32	0.20	0.17	0.28	0.09	0.23	0.25	0.11
126	0.13	0.11	0.13	0.11	0.09	0.19	0.03	0.11	0.11	0.05
167	0.07	0.12	0.10	0.05	0.09	0.08	0.03	0.16	0.09	0.04
156	0.01	0.04	<0.02	0.03	0.02	0.04	<0.01	<0.01	0.02	0.02
180	0.12	0.19	0.13	0.18	0.17	0.18	0.04	0.11	0.14	0.05
169	<0.02[a]	<0.02	<0.02	<0.02	<0.01	<0.02	<0.01	<0.02	ND	–
170	0.02	0.03	0.05	0.03	0.03	0.06	0.01	0.03	0.03	0.01
194	<0.01	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01	<0.01	ND	–
TEQ	0.013	0.011	0.014	0.011	0.009	0.019	0.003	0.012	0.012	0.005

[a] Limit of detection.

their mean levels in liquid soybean milks were between 4.2- and 2.7-times lower than those reported for soybean infant formulas. In addition, the standard deviations (S.D.s) calculated for this group of PCBs showed that variability among brands was lower for liquid-like milks (in the 0.47–0.11 range) than that found for soybean infant formulas (in the 2.06–0.85 range) [9].

Among the congeners detected, the lowest mean concentrations corresponded to PCBs 156, 170, 151, and 167 (in the 0.02–0.09 ng/g of lipids range, and with S.D. in the 0.01–0.08 range); whereas in soybean infant formulas previously analysed the lowest levels corresponded to PCBs 105, 167, 156 and 194, with levels of 0.13, 0.12, 0.05 and 0.03 ng/g of lipids, respectively. These results and those quoted above made apparent that some differences existed between the PCB profiles found for liquid soybean milks and those previously reported for soybean infant formulas. On the other hand, this mean PCB profile from liquid soybean milks was also different from those previously published for dairy products [6,11,15]. As usual in biological samples [16,17] PCBs 101, 153 and 138 were found to be the most abundant in cow's milks from Spain [11], whereas among the congeners detected the minor contributions corresponded, in decreasing order, to PCBs 167, 156 and 105. Therefore, the most apparent differences between cow's milks and liquid soybean milks were the largest contributions found in these latter for PCBs 77 and 126. Nevertheless, these high proportions of both non-*ortho*-substituted congeners agreed with those found when analysing soybean infant formulas [9]. Due to the high toxicity of the PCBs 77 and 126 [1] levels detected for these congeners in the analysed samples are cause of concern, especially because there were no obvious differences among the PCB profiles of the eight different liquid-like milk brands investigated.

In order to evaluate the toxicity of each sample studied, TEQs from PCBs were calculated following the toxic equivalent factor model proposed by the WHO [1]. Values calculated for all investigated brands (Table 1) ranged from 0.003 ng/g fat mass (brand 7) to 0.020 ng/g fat mass (brand 6). The total mean for the eight brands analysed was 0.012 ng TEQ/g fat mass. This mean value was 3.5-times lower than that previously reported for soybean infant formulas [9]. In spite of the largest proportion of the PCB congeners 77 and 126 found in these samples, this mean TEQ value was also lower than those usually found in literature for cow's milk [11].

3.2. PCB analysis in powdered soybean milks

Results corresponding to the PCB analysis in four different powdered soybean milks from three brands available in Spain are summarised in Table 2. All the investigated congeners were detected at quantifiable levels in any of the analysed samples, except for PCBs 105, 169 and 194, which were in all cases below their limits of detection. PCBs 28, 101 and 77 were found to be the most abundant in powdered soybean milks, with 2.41, 1.48 and 1.26 ng/g of lipids as mean concentrations, respectively. The S.D.s for this group of congeners were in the 0.53–0.73 range. In general, the following more abundant congeners were 153, 138 and 118, with mean levels of 0.50, 0.28 and 0.21 ng/g of lipids; whereas the lowest mean concentrations corresponded to PCBs 156, 170, 151 and 167 (0.01, 0.05, 0.06 and 0.06 ng/g of lipids as mean, respectively).

Some differences existed among the PCB levels detected in powdered soybean milks Nos. 1 and 2 (Table 2). Both samples were purchased from the same brand, and their lipid contents according to the manufacturer were 15.4 and 22.0%, respectively. The nature of the lipids added to the milks was not specified, but in all cases levels of the investigated PCBs were higher in sample 1 than in sample 2.

PCB profiles of the powdered soybean milks analysed were different from either those corresponding to liquid soybean milks or to soybean infant formulas [9]. In fact, powdered soybean milk profiles were more similar to those usually reported for biological [15,16] or cow's milk samples [6,11,15], than to those from the rest of the soybean derivatives studied.

TEQs from PCBs corresponding to each investigated powdered-like milks were calculated (Table 2). Very similar TEQ levels were found for the 4 samples (S.D.=0.003), and total TEQ mean (0.014 ng/g of lipids) was very closed to that calculated for liquid soybean milks.

Table 2
Results of the analysis of four different soybean powdered milks (ng/g of lipids)

PCB	Sample 1	Sample 2	Sample 3	Sample 4	Mean	S.D.
28	2.83	1.61	2.03	3.19	2.41	0.72
101	2.06	1.14	0.61	2.09	1.48	0.73
77	1.98	1.26	0.72	1.10	1.26	0.53
151	<0.03[a]	<0.02	0.26	<0.04	0.06	0.13
118	0.22	0.14	0.21	0.25	0.21	0.05
153	0.55	0.36	0.45	0.66	0.50	0.13
105	<0.02	<0.01	<0.01	<0.02	ND	–
138	0.29	0.19	0.30	0.32	0.28	0.06
126	0.14	0.10	0.11	0.17	0.13	0.03
167	0.11	0.08	<0.01	0.03	0.06	0.05
156	<0.02	<0.01	0.03	<0.02	0.01	0.02
180	0.15	0.11	<0.01	0.19	0.11	0.08
169	<0.02	<0.02	<0.02	<0.03	ND	–
170	0.08	0.06	<0.01	0.05	0.05	0.03
194	<0.02	<0.01	<0.01	<0.01	ND	–
TEQ	0.015	0.011	0.011	0.017	0.014	0.003

Symbols as in Table 1.

4. Conclusions

In recent years there has been an increase in the consumption of soybean products for being a good alternative to animal protein for people allergic to animal whey proteins. Some efforts have been made to ascertain the nutritional properties of these products. Nevertheless, the information concerning pollutant levels such as PCBs in soybean derivatives is still scarce. This is a cause of concern especially in the case of dairy-like products due to their wide consumption by population at large.

This paper shows that some differences were found among the PCB levels and, subsequently, among the TEQs calculated for the dairy-like products investigated. In general, lower mean levels with a lower variability among brands were found for liquid soybean milks than for powdered soybean milks.

On the other hand, some differences also exist among PCB levels and profiles from soybean dairy products and those usually reported for cow's dairy products. In general, PCB levels detected in the former were lower than those found in the latter, except for congeners 77, specifically, and 126, which were less concentrated in cow's milk. This was a common feature in different brands of soybean liquid and powdered milks and in the infant formulas [9] investigated. In spite of these higher levels of the toxic congeners 77 and 126, TEQs calculated for liquid and powdered soybean milks were lower than those usually reported in literature for cow's milk.

Acknowledgements

Authors thank the Comunidad Autónoma of Madrid for financial support (project COR 0035/94). They also thank Dr. M.J. González for providing PCB standards.

References

[1] H.G. Ahlbrog, G.C. Becking, C.S. Birnbaum, A. Brouwer, H.J.G.M. Dreks, M. Feeley, G. Golor, A. Hanberg, G.C. Larsen, A.K.D. Lieh, S.H. Safe, C. Schlatter, F. Waern, M. Younes, E. Yrjanheikki, Chemosphere 28 (1994) 1049.
[2] P. de Voogt, D.E. Well, L. Reutergardh, U.A.Th. Brinkman, Int. J. Environ. Anal. Chem. 40 (1990) 1.
[3] C.S. Hong, J. Xiao, A.C. Casey, B. Bush, E.F. Fitzgerald, S.A. Hwang, Arch. Environ. Contam. Toxicol. 27 (1994) 431.
[4] R. Duarte-Davidson, S.J. Harrad, Sc. Allen, K.C. Jones, Chemosphere 25 (1992) 1653.
[5] A. Schecter, P. Fürst, Ch. Fürst, H. Meemken, W. Groebel, D.Q. Vu, Chemosphere 19 (1989) 913.

[6] L.M. Hernández, M.A. Fernández, B. Jiménez, M.J. González, J.F. García, Bull. Environ. Contam. Toxicol. 52 (1994) 246.
[7] J. Mes, W.H. Newsome, Food Addit. Contam. 6 (1989) 365.
[8] M.C. García, M. Torre, M.L. Marina, F. Laborda, Crit. Rev. Food Sci. Nutr. 37 (1997) 361.
[9] L. Ramos, M. Torre, F. Laborda, M.L. Marina, J. Chromatogr. A, (1998) in press.
[10] K. Ballschmiter, M. Zell, Fresenius Z. Anal. Chem. 320 (1980) 323.
[11] L. Ramos, L.M. Hernández, M.J. González, Int. J. Environ. Anal. Chem. 67 (1998) 1.
[12] L. Ramos, L.M. Hernández, M.J. González, J. Chromatogr. A 759 (1997) 127.
[13] M.J. Gonzalez, L. Ramos, L.M. Hernanadez, Bull. Environ. Contam. Toxicol. 54 (1995) 349.
[14] B.R. Larsen, J. High Resolut. Chromatogr. 18 (1995) 141.
[15] M. Hippelein, H. Kaupp, G. Dörr, O. Hutzinger, Chemosphere 32 (1996) 1617.
[16] P. Dahl, G. Lindström, K. Wiberg, C. Rappe, Chemosphere 30 (1995) 2297.
[17] J.C. Duinker, A.H. Knap, K.C. Binkley, G.H. van Dam, A. Darrel-Rew, Mar. Pollut. Bull. 19 (1988) 74.