Enantiomeric composition and isomeric-specific PCB determination in dairy products from three different species: cow, sheep and goat

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INTRODUCTION

Although their use has been banned years ago, polychlorinated biphenyls (PCBs) are still a group of contaminants of great environmental concern. Due to their persistence and lipophilic character, they tend to concentrate in the food chain, particularly associated with fat. The ingestion of contaminated food is the principal pathway of human exposure, and in particular, foodstuffs of animal origin[1,2]. Of the 209 possible PCB congeners, 78 show axial chirality in their non-planar conformations. Nineteen out of those 78 were predicted to exist as stable atropisomers at room temperature[3], and nearly all are present in commercial PCB mixtures. As atropisomeric PCBs were released into the environment as racemates, enantioselective analysis potentially provides a more comprehensive understanding of the bioaccumulation and/or metabolism of those compounds. Moreover, some studies point to different biological and toxic behaviour of each of the enantiomers[4].

In the present study, the concentration of the most relevant PCB congeners and the enantiomeric fraction (EF)[5] of 11 chiral PCBs was determined in milk and dairy products made of milk from three different species (cow, sheep and goat). Heart-cut multidimensional gas chromatography was employed for the unambiguous determination of both enantiomers, using two different chiral columns, i.e., Chirasil-Dex and BGB-172. The aim of the study was to assess the enantiomeric composition of the dairy samples and to evaluate the possible differences between species and between the dairy products.

MATERIALS AND METHODS

Sampling

Whole milk and cheese samples from three different species, namely cow, sheep and goat, were analysed. Cheeses were commercially available and in order to assure that they were made from pure milk from each species, they were all under the Spanish "*Denominación de origen*" (Protected Denomination Origin - PDO) that imposes strict controls on the elaboration of the cheeses, including the type of milk used in the procedure. PDO *Manchego, Zamorano* and *Roncal* cheese were made of sheep milk, PDO *Palmero, Majorero, Ibores* of goats' milk and, PDO *San Simon, Arzúa-Ulloa* and *Mahon* of cows' milk. A total of 9 milk samples from the three different species were included in the study. Cows' milk was commercially available. Sheep and goats' milk samples were obtained from different farms around Madrid.

Sample preparation

Milk samples were freeze-dried prior to analysis. Sample extraction consisted of a matrix solid phase dispersion of the sample with anhydrous sodium sulphate and silica gel, using acetone:hexane (1:1, V/V) as elution solvent. Further clean-up steps were performed using activated and modified multilayer silica columns[6]. No further fractionation has been carried out.

Instrumental analysis

GC-ECD analysis

The list of PCBs analysed included non- and mono-*ortho* substituted PCBs, the set of seven indicators usually used for monitoring purposes, as well as those chiral congeners present in commercial PCB mixtures and food samples (PCB 28, 52, 77, 84, 91, 95, 101, 105, 114, 118, 123, 126, 132, 135, 136, 138, 149, 153, 156, 157, 167, 169, 171, 174, 176, 180, 183 and 189). Analyses were carried out on a Varian 34000 CX GC (Palo Alto, USA) equipped with a BPX-5 column (60m × 0.25 mm I.D., 0.25μm film thickness). Injector and ECD detector temperatures were 280ºC and 300ºC, respectively. Nitrogen was used as carrier gas. Extracts were injected in splitless mode (splitless time, 1min). The oven temperature program was as follows: 80ºC (1min), at 30 ºC/min to 185 ºC (3min), at 1.9 ºC/min to 234 ºC (65.5min), at 2 ºC/min to 270 ºC.

Heart-cut Multidimensional gas chromatography (Heart-cut MDGC)

Enantiomeric composition of chiral PCBs was determined using a Heart-cut MDGC system (Varian) equipped with two independent ovens. Column switching was achieved by means of a Deans valve placed in the first oven. Transfer line was maintained at 280ºC. An achiral DB5 column (30m × 0,25mm I.D., 0.25μm film thickness, J&W Scientific, USA) was used as pre-column in the first oven. In the second oven, two chiral columns, both a Chirasil-Dex (2,3,6-tri-*ortho*-methyl β-CD, 25m × 0.25mm I.D., 0.25μm film thickness, Varian-Chrompack, Middelburg, The Netherlands) and a BGB-172 (25% 2,3,6-tert-butyldimethylsilyl β-CD, 30m × 0.25mm I.D., 0.18μm film thickness, BGB Analytik, Adliswil, Switzerland), were used as main columns. Injections were performed at 270ºC in the splitless mode (splitless time, 1min). Both ovens were equipped with a ^{63}Ni-ECD, maintained at 300 ºC and 250ºC for the monitor and main detector, respectively. Nitrogen was used as carrier gas. The oven temperature programs were as follows: First oven

(DB-5): 80ºC (1min), at 30ºC/min to 185ºC (3min), at 1.9ºC/min to 234ºC (25min), at 2ºC/min to 270ºC. Second oven (Chirasil-Dex): 100ºC (1min), at 5ºC/min to 160ºC (30min), at 1ºC/min to 170ºC (10min), at 1ºC/min to 180ºC; Second oven (BGB-172): 90ºC (1min), at 15ºC/min to 170ºC (25min), at 2ºC/min to 180ºC (30min), at 2ºC/min to 200ºC.

RESULTS AND DISCUSSION

Total PCB concentration in milk samples ranged from 3.55 to 39.3 ng/g on a lipid weight basis (l.w.), with concentrations in cows' milk exceeding those in than sheep and goats' milk samples. Concentrations found in cheese ranged from 11.0 to 30.0 ng/g l.w. Concerning coplanar PCBs, in most of the samples, non-*ortho* PCBs were not detected, and mono-*ortho* PCBs accounted for less than 18% to the total PCB content. Congener specific profile showed PCBs 138, 153 and 180 as the most abundant, followed by congeners 52 and 95. Among coplanar congeners, PCB 118, followed by 105, were the most abundant ones.

For the proper determination of the enantiomeric composition in the food samples analysed, two chiral columns were used: Chirasil-Dex for the determination of PCBs 91, 95, 132, 135, 136, 149, 174, 176, and BGB-172 for the determination of PCBs 84, 171 and 183. Results revealed that 6 out of the 11 chiral PCBs investigated (PCBs 84, 91, 95, 132, 149 and 174) showed a racemic or nearly racemic composition in all samples. Among all atropisomers, PCBs 95, 132 and 149 are the ones presenting the highest concentrations and those usually found at higher levels in food samples. PCB 135 and 136 were found at low levels, and both showed EF below 0.5 with the first enantiomer being slightly depleted, except for PCB 135 in PDO *Mahón* (cow cheese) where the first eluted enantiomer was enriched (EF=0.90). In the case of PCB 183 and 171, non-racemic composition in some of the samples analysed was observed (**Figure 1**). PCB 171 was found to have a nearly racemic composition in cows' milk samples, while strong deviations from racemic were found for sheep's milk that showed a higher enantio-enrichment of the first eluting enantiomer (EF=0.70-0.82). Regarding goats' milk no agreement between the two samples analysed was observed (EF=0.15-0.77). With respect to cheese samples, cows'milk cheese showed a racemic composition similar to that observed for the corresponding milk. Sheep's cheese revealed an enantio-enrichment of the second eluting enantiomer (EF=0.37-0.46), that was opposite to that observed for the corresponding milk, while the two goats' cheese samples were of racemic composition.

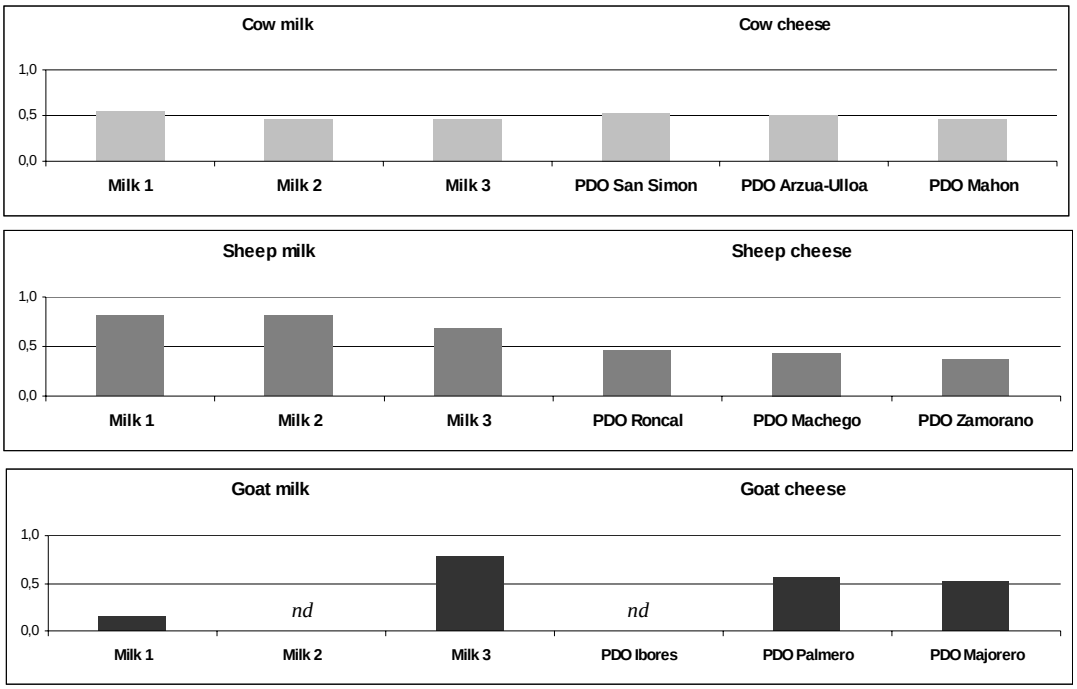

Figure 1. Enantiomeric fractions (EFs) of PCB 171 in the milk and dairy samples under study.

Regarding PCB 183, cows' milk showed again a nearly racemic composition (EF=0.48), while sheep and goats' milk presented an enrichment of the second eluting enantiomer in all samples (**Figure 2**). Concerning the cows'milk cheese samples, no remarkable alteration of the EF was found compared to those obtained in the corresponding milk. For sheep's milk cheese samples and in the same way as for the corresponding milk, this congener presented an enrichment of the second eluting enantiomer (EF=0.42-0.45), but not as remarkable as in the milk samples. Concerning goats' cheese samples, EFs ranged from 0.48 to 0.50. This near-racemic composition is in contrast to the enrichment of the second eluting enantiomer in the corresponding milk samples.

Chiral analysis has been used in this study to provide results on the enantiomeric composition of several PCBs in milk and dairy samples. Results revealed a non-

racemic composition of PCB 171 and 183 in sheep and goats' milk samples compared to cows' milk, as well as in sheep and goats' milk cheese. Although PCB 183 has been regarded as a difficult to metabolise congener because it lacks vicinal hydrogen atoms in *meta*/*para* and *ortho*/*meta* positions[7], the results obtained suggested that these two species accumulate and/or metabolise this congener enantioselectively.

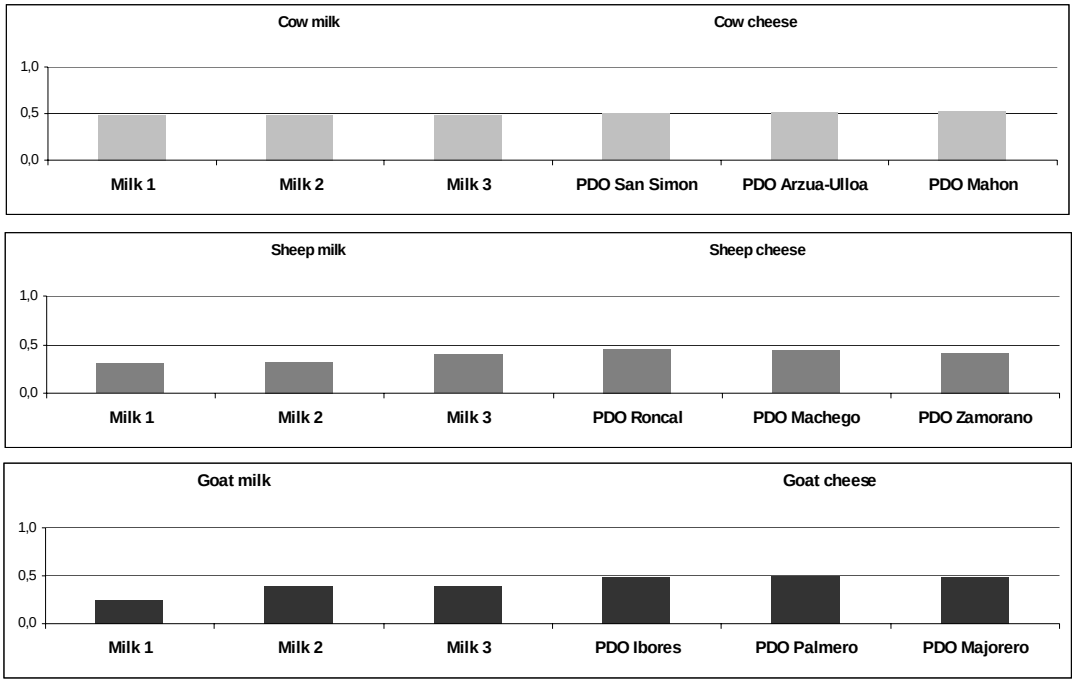

Figure 2. Enantiomeric fractions (EFs) of PCB 183 in the milk and dairy samples under study.

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