REMOVAL OF PCDDs/Fs AND BENZO(a)PYRENE IN EDIBLE VEGETABLE OILS USING ACTIVATED CARBON

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Introduction

PCDDs and PCDFs belong to the well-known group of persistent organic compounds (POP), commonly called dioxins. These compounds have been detected in vegetable oils[1] and are considered as undesirable substances. Therefore Regulation (EC) 2375/2001 in force since July 2002 and subsequent modifications establish the maximum contents accepted in human food, being 0.75 pg/g lipid weight for vegetable oils. Recently these limits have been modified by Regulation (EC) 199/2006, which add PCB 'dioxin like' in their estimation.

Bunge Ibérica S.A-La Coruña is dedicated to the activity of crushing and refining oilseeds. According to its strategy of productive improvement and to HACCP criteria, it has developed the present study for removing undesirable substances in edible vegetable oil. This study has been supported by Xunta de Galicia (Project PGDIT03TAL19E).

Bleaching with earths is a common practice applied in the refining of vegetable oils[2-4]. Since 2005 Regulation (EC) 208/2005 establish a limit value of 2.0 µg/kg of benzo(a)pyrene for fat and oil for direct human consume. However Bunge Ibérica S.A. has successfully used activated carbon as a specific adsorbent for removing benzo(a)pyrene during refining of crude soybean oil since 2001. The present study was conducted to compare the efficiencies of different types of activated carbon, with different physical-chemical properties (internal surface area, particle size, etc.) for removing PCDDs, PCDFs and benzo(a)pyrene jointly.

Materials and Methods

Bleaching Tests

Bleaching or clarification reactions were performed in a jacketed reaction vessel with different necks for: mechanical stirring, vacuum pump connection, temperature control and addition of activated carbon and bleaching earths. Neutralized soybean oil was used as reaction media. After clarification reactions the oil was filtered under vacuum with Albet FV-A glass fibre filters (Sant Boi de Llobregat, Spain) before analysis.

Analysis of Benzo(a)pyrene

Benzo(a)pyrene analysis was based on ISO 15302 and IUPAC 2.608 method. The oil sample was dissolved in hexane and an aliquot was cleaned-up using alumina columns and hexane. The hexane was removed by rotary-evaporation and replaced with acetonitrile. Analysis was performed by HPLC. An Agilent 1100 (Agilent Technologies, Bellefonte, USA) equipped with a Vydac C18 201TP52 column (250 mm x 2.1 mm i.d., 5 µm particle size; Hesperia, USA) and a fluorescence detector was used. Benzo(a)pyrene were eluted in isocratic mode (88% acetonitrile : 12% water) and detected in programmed fluorescence mode.

Analysis of PCDDs/Fs

Oil samples were spiked with labelled PCDDs/Fs standards (EPA 1613-LCS, Wellington Laboratories, Ontario, Canada) and then dissolved directly in n-hexane. The fats were removed by treatment with sulphuric acid to obtain a successful clean-up.

Clean-up was performed on the Power Prep FMS system (Fluid Management Systems, Waltham, USA). Procedure is based on solid/liquid adsorption chromatography using a set of disposable columns: multilayer silica columns, basic alumina columns and PX-21 carbon columns. A final extract in toluene was recovered. This extract was concentrated

up-to 2 mL using a rotary evaporator (Büchi, Flawil, Switzerland) and the remaining solvent was removed with a gently stream of N_2. Before HRGC/HRMS analysis, samples were rebuilt with the recovery standards: 5 μL of EPA 1613-ISS (Wellington Laboratories, Ontario, Canada) plus 10 μL of nonane.

PCDDs/Fs analysis was based on US EPA Method 1613. A MAT 95 XP coupled to a Trace GC 2000 series gas chromatograph (Thermo Electron, Bremen, Germany) equipped with a CTC GC PAL autosampler (CTC Analytics, Zwingen, Switzerland) was used. Analysis was performed using a DB-5 capillary column (30 m x 0.25 mm x 0.25 μm; Agilent Technologies, Bellefonte, USA) with helium as carrier gas at 1 mL/min in the splitless injection mode (2 μL). The temperature program was from 140ºC (2 min) to 200ºC at 11ºC/min, and then to 300ºC at 3 ºC/min. Transfer line temperature was set at 290ºC. The mass spectrometer was operated in EI mode (45 eV), using multiple ion detection (MID). Source temperature was set at 260ºC and the spectrometer was tuned to a minimum resolution of 10,000 (10% valley) using FC-43. The two most abundant isotope peaks (M^+ and $[M+2]^+$ or $[M+4]^+$) of each PCDDs/Fs congener were used.

Identification was carried out using chromatographic retention times and isotopic ratios. Quantification was achieved by isotopic dilution method using relative response factors (RRF) obtained by analysis of standard solution mixtures (EPA 1613 CVS solutions; Wellington Laboratories, Ontario, Canada).

Results and Discussion

Reaction media was prepared with neutralized soybean oil spiked with a known concentration of the required compounds using commercial standard solutions: benzo(a)pyrene (10 ng/μL in acetonitrile; Dr. Ehrenstorfer, Augsburg, Germany) and PCDDs/Fs (NK-ST-A in nonane; Wellington Laboratories, Ontario, Canada). The final concentration was determined with the proper analytical technique for each compound.

The investigation undertakes three different aspects:

Kinetic Experiments

The kinetic experiments were carried out following the decrease in the content of benzo(a)pyrene in samples obtained at different times. These experiments were carried out with different types of activated carbon at a concentration of 0.03% in the oil. Characteristics of the different sorbents are shown in Table 1. The aim of these experiments was to establish the necessary contact time between sorbent-adsorbate for reducing the level of contaminant under the legal limit. Figure 1 shows experimental data of benzo(a)pyrene (ppb) concentration versus time (min). Fractional degree of removal (%DR) was calculated at reaction time of 25 min (real Residence Time for bleaching step in the industrial refining process) to compare the effectiveness of the different activated carbons. These values are also shown in Table 1.

Table 1. Physico-chemical properties of different types of activated carbon based on manufacturer's data. Fractional degree of removal (DR) expressed as percentage.

	BET surface area (m^2/g)	Particle size (μm)	Iodine index (mg/g)	DR (%) at 25 min
C1	*	15-35	1,000-1,100	93.78
C2	650	With > 150 (4%)	750	83.45
C3	1,000	With < 75 (>90%)	950	81.70
C4	*	*	*	54.70

(*): not available.

| Time (min)	C1 [B(a)P] (ppb)	C2 [B(a)P] (ppb)	C3 [B(a)P] (ppb)	C4 [B(a)P] (ppb)
0	7.07	5.74	9.29	9.67
2	1.03	1.76	2.86	6.18
5	0.72	1.42	2.55	6.16
10	0.61	1.22	2.04	5.25
15	0.57	1.15	1.82	5.05
20	0.45	0.95	1.90	4.89
25	0.44	0.95	1.70	4.56
30	0.32		1.76	4.38
35	0.31		1.68	4.38
40	0.33		1.73	3.16
50	0.27		1.51	3.16
60	0.27		1.55	3.03
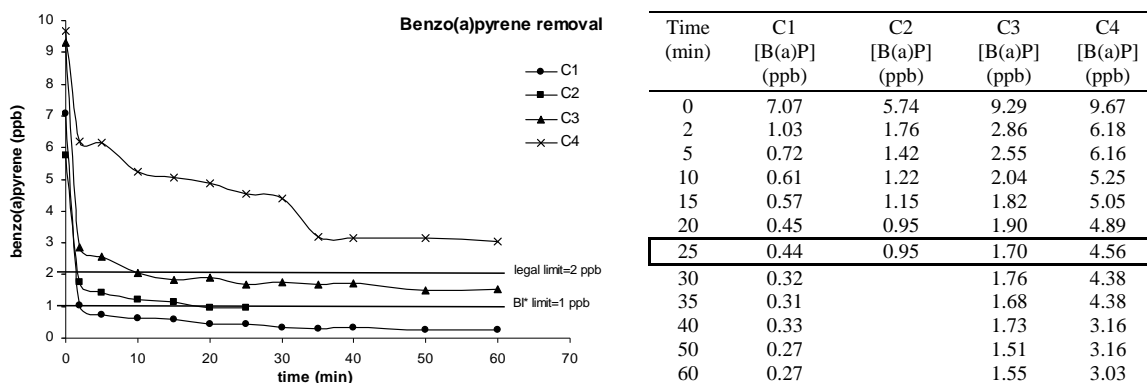
Figure 1. Kinetic of benzo(a)pyrene removal with different activated carbons (Reaction Conditions: Reaction Tª: 100-105ºC; Reaction media: 1,500 g of neutralized soybean oil; Adsorbent mixture composed of Bleaching Earth: Activated Carbon (93:7); Percentage of activated carbon in the oil: 0.03%). (*) BI stands for Bunge Ibérica S.A.

Adsorption Experiments for Benzo(a)pyrene Removal
Experiments were carried out by measuring the content decrease of benzo(a)pyrene using different percentages of activated carbon in the oil. The applicability of three types of isotherms, namely Linear, Langmuir and Freundlich isotherms, was investigated. Figure 2 shows the non-linear fit of experimental data to the Langmuir equation. Isotherm parameters were obtained for characterizing the interaction between adsorbent-adsorbed substrate.
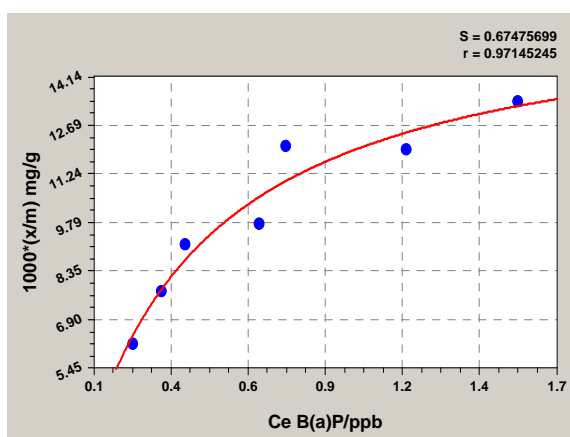
Langmuir equation : $\dfrac{x}{m} = \dfrac{C_e \cdot a \cdot b}{1 + b \cdot C_e}$

where:

x/m: adsorbed substrate (benzo(a)pyrene) in mg per adsorbent (activated carbon) in g.

Ce: equilibrium concentration of B(a)P in ppb.

a: constant related to the area occupied by a monolayer of adsorbent substrate. It's a measure of sorption capacity (mg/g).

b: It's a measure of the intensity of sorption process (g/μg).

In this case: a: 0.016 mg/g, b: 2.65 g/μg

Figure 2. Curve fit of experimental data to Langmuir equation (Reaction Conditions: Reaction Tª: 100-105ºC; Reaction Time: 60 min; Reaction Media: 1,592 g of neutralized soybean oil with 2.14 ppb of benzo(a)pyrene; Adsorbent mixture composed of Bleaching Earth:C2 (70:30); Variation of percentage of activated carbon in the oil: 0.004-0.03%).

Adsorption Experiments for PCDDs/Fs Removal
Experiments were carried out by measuring the content decrease of PCDDs/Fs using different percentages of activated carbon in the oil. The applicability of the same types of isotherms (Linear, Langmuir and Freundlich isotherms) was also investigated. However, good correlations could not be drawn conclusively. Figure 3 shows the plot of PCDDs/Fs concentration (expressed as pg/g and WHO-TEQ pg/g) *vs.* percentage of carbon.
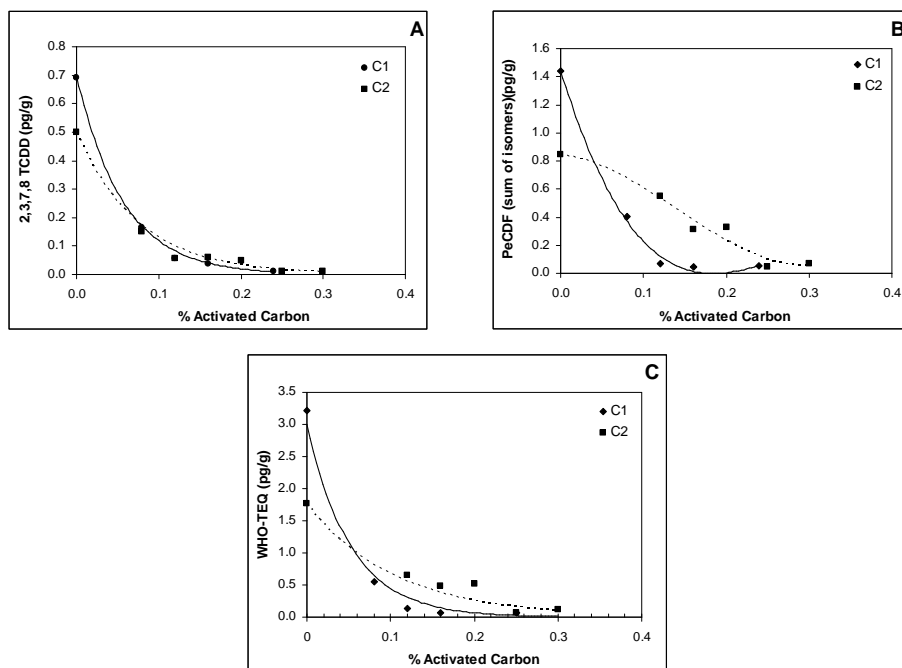
Figure 3. Comparison of 2,3,7,8-TCDD removal with C1 and C2 (a); comparison of ΣPeCDF removal with C1 and C2 (b); comparison of WHO-TEQ (pg/g) removal with C1 and C2 (c).

As it can be seen in the kinetics results, a percentage of 0.03% is enough to reduce the benzo(a)pyrene content under the legal limit, and even the internal limit, for C1. Using C2 yields a worse result. Nevertheless this is insufficient for removing dioxins when concentration ranges from 1.76 to 3.2 ppt (WHO-TEQ). This represents 2 and 4 times the legal limit (0.75 pg/g lipid weight) in raw material. Percentage of 0.1-0.15% ensures an efficient removal of benzo(a)pyrene and PCDDs/Fs even in these conditions. Removal efficiency of both carbons was compared in terms of fractional degree of removal (%DR) at a cost-efficient carbon percentage of 0.12% (Table 2).

Table 2. Removal efficiency of C1 and C2 at a percentage of 0.12% in terms of fractional degree of removal (%DR).

	DR (%) 2,3,7,8-TCDD	DR (%) ΣPeCDF	DR (%) WHO-TEQ
C1	91.90	95.14	95.65
C2	89.06	34.12	63.07

Acknowledgements

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