DIOXINS, FURANS AND DL-PCBs IN BIOTECHNOLOGICALLY PRODUCED OMEGA-3 FATTY ACIDS

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Introduction

Omega-3 polyunsaturated fatty acids (PUFA) have beneficial effects on human health, reducing the risk of cardiovascular and hypertension diseases, preventing from cancer, Alzheimer or schizophrenia[1,2]. The recommended daily intake (RDI) for humans ranges between 450-650 mg/day for a combination of the two most important omega-3 PUFAs: eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6). Specific needs for DHA are higher during pregnancy and infant age due to its importance for an early human development. PUFAs are also used in aquaculture industry as feed additive, since they are essential nutrients for cultured marine fish.

Production of PUFAs has been traditionally based on fish oil, which is a by-product of the fishmeal manufacturing industry[1]. However, fish oil can present relatively high concentrations of ubiquitous contaminants in marine ecosystems[3-9]. Dioxins, furans and PCBs bioaccumulate in the fatty tissues of organisms due to their lipophilic nature, especially in those of marine animals, which present low metabolic capability. The consumption of PUFAs is therefore coupled with an increased intake of pollutants, and an exposure to their adverse health effects. In addition to contamination, using fish oil as a PUFA source is susceptible to composition variability and quality, and it is becoming unpopular for unpleasant smell and taste, poor oxidative stability and expensive purification. On the other hand, fish oil price is experiencing rapid increase due to a flat supply and increased global demand for this commodity. FAO predicts that fish oil demand in 2015 will reach 145% of the historical global production capacity[1].

Such situation settles the need for an alternative production of fatty acids. Within the biotechnological field, different marine microorganisms, such as yeasts and microalgae, present the ability to grow on waste carbon sources, and naturally produce PUFAs by fermentation, and other high value-added products (carotenoids, terpenes, organic acids, polyhydroxyalkanoates)[2]. Among all these marine microorganisms that generate PUFAs, photosynthetic ones present operative limitations: the need of light determines a complex design and a restricted productivity. As a consequence, production and maintenance of photosynthetic biomass turns out to be a 70% more expensive than heterotrophic biomass. Conversely, the heterotrophic microalgae *Aurantiochytrium sp*. was proved to be an excellent PUFA producer with ability to metabolize crude glycerol[10]. The triglycerides or fatty acids can be extracted from the microorganisms, and then added in fortified foods, or alternatively the biomass can be directly used as a feed additive in various animal industries[1]. Moreover, a crude glycerol-based microalgae mass culture industry would mean a double opportunity to valorize an industrial byproduct and, at the same time, to economize the production of valued-added goods.

The aim of this study is to evaluate the pollutant levels of omega-3 fatty acids produced by microalgae, which have been grown using crude glycerol. The analytes targeted are polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (PCBs).

Materials and methods

Algal strain, medium and culture conditions

The strain ATCC MYA-1381 of *Aurantiochytrium limacinum* was used. The cells were grown in 30 g/l of carbon source (crude glycerol), ca. 1 g/l yeast extract and ca. 1 g/l tryptone in artificial seawater[11].

Crude glycerol was obtained from alkaline-catalyzed transesterification of used cooking oils (UCO) using methanol. Purity of crude glycerol was between 75-80%.

Biomass was grown in 1 L Erlenmeyer flask. The culture was kept at 20ºC and 150 rpm of orbital agitation and left for three days. Subcultured cells were used as inoculum.

Samples

The samples analyzed were: oil sample obtained from biomass grown in crude glycerol, crude glycerol used as carbon source for the process, other organic components of the culture media than glycerol (yeast and tryptone) and inorganic species of the growing media. Additionally, laboratory blanks were also analyzed.

Analysis

Before analyzing the sample, lipids were extracted from biomass by ultrasound-assisted Bligh and Dyer method[12]. Mainly, biomass samples were homogenized with a ternary-solvent system of chloroform, methanol and water. Proportions were those that provided interaction with the sample, so a single phase was formed by means of combining miscible volumes. Chloroform, methanol and water were added in a ratio 1:2:0.4. The mixture was subjected to ultrasonic energy for ca. 50 min. Chloroform and water were added yielding a mixture of 3:2:1.4 chloroform-methanol-water. Ultrasound was applied for around 20 min after each addition. Two phases were then formed so that the organic one containing the lipids could be separated.

Glycerol sample and inorganic components of culture media were extracted with hexane after diluting them in water. Tryptone and yeast were extracted with toluene in a Soxhlet apparatus for 24 h.

After the extraction, each sample was cleaned-up in a multilayer silica column. From top to bottom, the column was filled with anhydrous sodium sulphate, sulphuric-modified silica (22% and 44%), activated silica, sodium hydroxide-modified silica, activated silica and silver nitrate-modified silica. PCDD/Fs and dl-PCBs were eluted with hexane from the column.

After the clean-up, PCDD/F and PCBs were separated in pre-packed SPE carbon tubes. Additional separation of dl-PCB from the bulk of PCBs was performed in an HPLC equipped with a pyrenyl column.

The fractions obtained were concentrated under nitrogen stream and analyzed by HRGC-HRMS, following EPA 1613 method. Quantification was carried out by the isotopic dilution method, based on the use of $^{13}C_{12}$-labelled PCDD/F and dl-PCB internal standards. For TEQ calculation, WHO-2005 TEFs were used.

Results and discussion

Biomass obtained from the process yielded ca. 49 % of lipids. DHA was about 20% of the dry cell mass.

The levels detected in biotechnologically-produced oil were 1.26 pg TEQ/g for PCDD/F, 2.23 pg TEQ/g for dl-PCB and 3.50 pg TEQ/g for the sum of PCDD/F and dl-PCB. These values are below the maximum levels established for human consumption (1.75 pg TEQ/g, for PCDD/F, and 6.0 pg TEQ/g, for PCDD/F+dl-PCB) or animal feed (5.0 pg TEQ/g, for PCDD/F, and 20.0 pg TEQ/g, for PCDD/F+dl-PCB)[13-14]. Therefore, from this perspective, this oil can be introduced in the market.

PCDD/F concentrations for biotech oil and different fish oils reported in literature are compared in Figure 1. A very broad range of concentrations can be detected in fish oils, mainly depending on the origin and on the refining process they have followed. Biotechnologically produced omega-3 oil, from crude glycerol and whitout any refining process, showed PCDD/F and dl-PCB concentrations similar or lower than most of the fish oil reported.

Although it is difficult to exactly establish the source of dioxins and PCBs in the biotech oil, crude glycerol and the other components of the culture medium (tryptone, yeast and inorganic components) were analyzed. For PCDD/Fs, tryptone, yeast and inorganic components showed most congener concentrations below LOQ and for crude glycerol only some congeners (1,2,3,7,8-PeCDF, 1,2,3,4,6,7,8-HpCDD and OCDD) were detected. For dl-PCBs, results were similar: for tryptone, yeast and inorganic components most concentrations were below LOQ. However, for glycerol most of them were detected. In Figure 2, the dl-PCB profile for biotech oil and crude glycerol are compared. The profiles of both samples were quite similar and characterized by PCB 118, 105 and 156, followed by PCB 77 and PCB 167. Since crude glycerol is the major component of the culture medium and the profiles were similar, the hypothesis that PCB levels detected in the oil came from glycerol seems to be plausible.

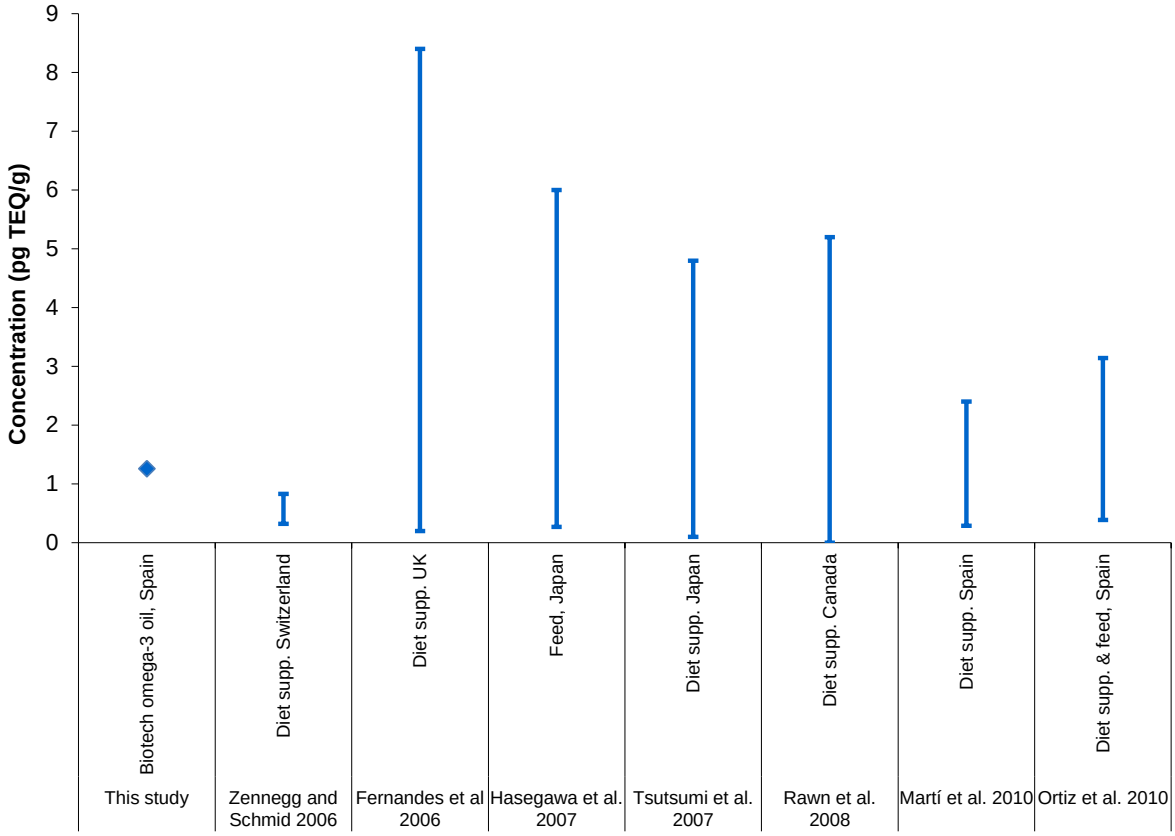

Figure 1. Comparison of PCDD/F concentrations of biotech omega-3 oil and different fish oils reported in literature.

In conclusion, levels of PCDD/Fs and dl-PCBs detected in biotechnologically produced omega-3 oil were similar or lower than those detected in fish oil and below the maximum levels established by regulations in force. Most of these pollutants could have their origin in the glycerol used as carbon source.

References

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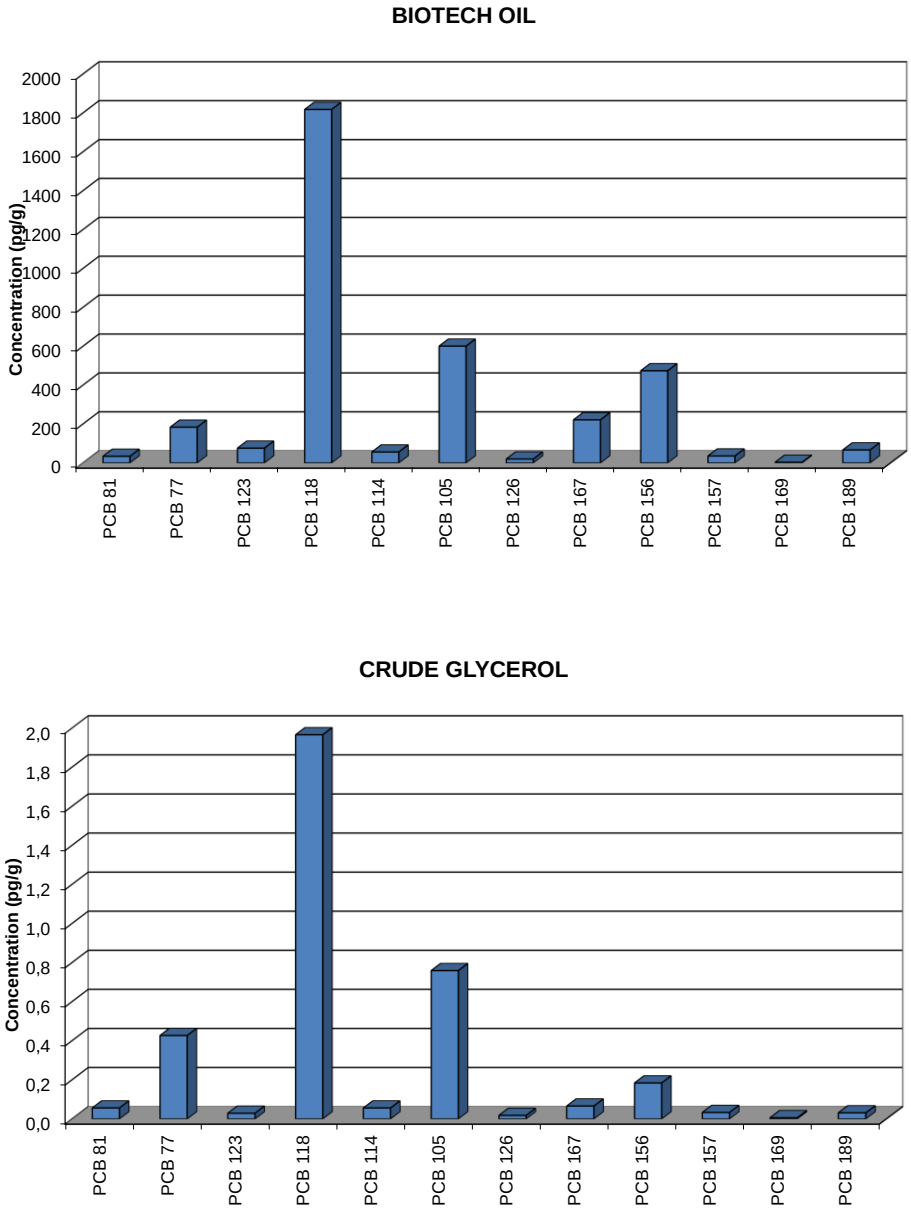

Figure 2. Profile of dl-PCBs for biotechnologically produced omega-3 oil and crude glycerol used as carbon source.