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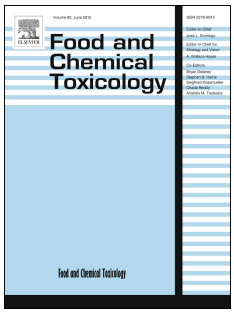

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PRELIMINARY ASSESSMENT OF GALAXOLIDE BIOACCESSIBILITY IN RAW AND COOKED FISH

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Abstract

Generally, dietary intake assessment and risk characterization are evaluated using contaminant concentration in raw fish while it is usually ingested cooked, which can cause an overestimation because one of the essential issues for risk-benefit analysis is to determine the maximum amount of a contaminant that can be released from the food matrix and be absorbed by the human body, called bioaccessibility. Moreover, despite most seafood products are cooked before consumption, risk assessment is still evaluated in raw products, strongly affecting public health guidelines. In the present study, an *in vitro* bioaccessibility assay was performed for Galaxolide (HHCB) in fish samples. Raw spiked hake samples were *in vitro* digested and aliquots of each fraction of the digestion process were analysed. HHCB was quantitatively present in the bioaccessibility fraction. The effect of fish cooking on HHCB was also evaluated in cod and mackerel samples. Results demonstrate that steaming and grilling processes lead to a loss of 50-70% HHCB in fish.

Keywords: *galaxolide, bioaccessibility, fish, steaming, grilling.*

1. Introduction

The emerging organic contaminant (EOC) Galaxolide (HHCB) is one of the most reported synthetic musk fragrances in the last years due to its presence in several environmental compartments (Marchal and Beltran, 2016). This compound has been extensively used as personal care product (PCP) and in daily consumer products such as perfumes, cosmetics, soaps, fabric conditioners, among others and has been included in the EPA's high production list (Organisation for economic co-operation and development, 2004). Because of its large industrial production and consumption, this compound is released into the environment, mainly through wastewater treatment plants (WWTPs), since they do not remove completely the input of HHCB (Marchal and Beltran, 2016).

Some characteristics of this polycyclic musk are the hydrophobic behaviour and poor water solubility. Thus, HHCB is expected to adsorb onto organic matter and lipids. In fact, HHCB is found in different environmental matrices, such as water and sediments (Homen et al.,2016; Cavalheiro et al., 2015; Godayol et al., 2015; Vallecillos et al., 2015a), as well as in aquatic biota (Saraiva et al., 2016; Trabalón et al., 2015; Cunha et al., 2015; Ziarrusta et al., 2014), including fish and shellfish. Although, some assessments concluded that HHCB does not represent a major risk concern for human health (EPA, 2016; HERA, 2004), several studies have demonstrated its ubiquity in fish and shellfish at high concentration levels (Marchal and Beltran, 2016; Trabalón et al., 2015; Cunha et al., 2015), as well as the toxic effects related to aquatic organisms (Kannan et al., 2005; Rainieri et al., 2017). For example, Trabalón *et al* (2015) reported concentrations for ten musk compounds and HHCB was the musk congener that showed the highest concentrations, being sardine (367.3 ng g^{-1} (d.w.)), mackerel (303.9 ng g^{-1} (d.w.)) and cod (146.7 ng g^{-1} (d.w.)) the main affected species. Moreover, Cunha *et* al (2015) reported high concentrations of HHCB for mussels, which ranged between 8.68 and 34.52 ng g^{-1} (d.w.) and for clams (33.10 ng g^{-1} (d.w.)). In addition, Mottaleb *et* al (2009) reported concentrations of HHCB between 234 and 970 ng g^{-1} expressed as wet weight (w.w.) for bluegrill. These values were 10 times higher than those reported for the other contaminants studied. For these reasons, risk assessment for human

health is one of the topics that is receiving an increasing interest. Although some studies have reported the relevance of dermal exposure (Correira et al., 2013) or inhalation exposure through indoor dust (Lu et al., 2011) as routes of PCPs entrance in the human body, dietary intake is considered as one of the main routes of exposure, especially through seafood consumption (Trabalón et al., 2015; Taylor et al., 2014). Nevertheless, limited available information exists for HHCB in seafood, being reflected in few studies addressing risk assessment via fish consumption for this compound (Trabalón et al., 2015).

In order to better understand the amount of contaminant that is released from the seafood matrix to the human body, it is necessary take into account different factors such as the type of food matrix, the physicochemical properties of the analyte, and the cooking process, among others. All these factors affect the bioaccessibility process, which is the fraction of an analyte that is released from the food matrix to the gastrointestinal tract during the digestion process (Cunha et al., 2017; Alves et al., 2017; Braga et al., 2016; Manita et al., 2017). Although bioaccessibility studies are scarce for EOCs, recently Alves *et al* (2017) performed a preliminary study evaluating bioaccessibility for some EOCs, including perfluorinated compounds (PFCs), brominated flame retardants (BFRs), among others, in raw and cooked seafood. In addition, Cunha *et al* (2017) assessed the bioaccessibility of bisphenol A in canned seafood. Yu *et* al (2011) also assessed the bioaccessibility of polybrominated diphenyl ethers (PBDEs) in different food stuffs from China, in order to understand the different contribution pathways of PBDEs through the diet to avoid misleading results of exposure assessment. Therefore, bioaccessibility analysis helps to refine risk assessment and guidelines for seafood consumption for authorities, industry and consumers.

On the other hand, in most studies found in scientific literature about the dietary intake of environmental contaminants, analyses are generally carried out only in raw food products (Martellini et al., 2016; Chang et al., 2017; Wang et al., 2011). However, it is clear that the vast majority of food products are consumed after cooking. Moreover, the combination of cooking treatments and seafood displays different behaviours according to the contaminant

(Domingo, 2016). For this reason, there is an urgent need to collect more information about how different cooking processes may affect contaminants.

The aim of this study is to validate for the first time an *in vitro* digestion assay to determine the bioaccessibility of HHCB, and to test two different cooking processes to understand whether they do affect HHCB levels.

2. Experimental

2.1. Standard solutions

The standard 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-[g]-2-benzopyran (HHCB, galaxolide) was supplied by Promochem Iberia (Barcelona, Spain). The internal standard d_{15}-musk xylene (d_{15}-MX) was purchased as a 100 mg L^{-1} solution in acetone from Symta (Madrid, Spain). Individual standard solutions of HHCB were prepared in acetone at concentration of 168 mg L^{-1} for the bioaccessibility experiments and at 4,000 mg L^{-1} for the cooking effect assessment, followed by a working solution of 1 mg L^{-1} prepared in ethyl acetate to enable the preparation of standard solutions for the calibration curve.

Acetone and ethyl acetate were GC grade with purity > 99.9%, supplied by Prolabo (VWR, Llinars del Vallès, Barcelona, Spain).

2.2. Sample pretreatment and culinary treatments

Three fish species were collected in different Portuguese markets: hake (*Merluccius merluccius*) used for the validation of the bioaccessibility assay; cod (*Gadus morhua*) and mackerel (*Scomber scombrus*) used in the cooking effect study. Hake was selected because of its low lipid content (2%) which facilitates the extraction procedure and analysis, while cod and mackerel were selected as representative species of low (1%) and high (17%) lipid content, respectively, because they showed higher concentrations of HHCB in the study of Trabalón *et al* (2015). Specimens of each species came from commercial retailers and had

uniform sizes and weights. For each species, the muscle tissue was collected from fillets without skin and pooled. In addition, cod and mackerel samples were divided into three portions, one for raw assessment and the others for cooking assessment (steaming and grilling).

Steaming and grilling were selected as the most common cooking processes for fish (Alves et al., 2017; Domingo, 2016; Aznar-Alemany et al., 2017), in order to understand whether they affect the levels of HHCB. Steaming was carried out at 105ºC during 15 min for fish wrapped in aluminium foil whereas grilling, a kitchen electric grilling device was used to cook the fish during 10 min at 175ºC. Species with different lipid content were selected in order to asses not only the effect of the culinary procedure but also the effect of lipids in HHCB degradation. Since previous studies showed the presence of HHCB at higher levels in these species, non-spiked samples were used. For each species, pooled samples were divided in three groups (raw, grilled and steamed) and each group was divided in three subgroups that were analysed in triplicate.

All raw and cooked samples were homogenised with a grinder (Retasch Grindomix GM2000, Germany) using polypropylene cups and stainless-steel knives at 5,000 rpm until complete visual disruption of the tissues. Finally, raw samples were kept at -20ºC until being used. Solid samples for the chromatographic analysis were lyophilised using a freeze-drying system (Labconco, Kansas City, MO, USA) and crushed using a mortar and pestle, being kept in a dry place until analysis.

In order to quantify the effect of the cooking process on HHCB concentration, recoveries were calculated by the following equation 1:

$$R = \frac{[HHCB]_{cf} x 100}{[HHCB]_{rf}} \qquad (1)$$

where *R* is the recovery of the HHCB in each culinary treatment for different fish species, $[HHCB]_{cf}$ means the concentration of HHCB in cooked fish samples (ng g^{-1}) and $[HHCB]_{rf}$ means the concentration (ng g^{-1}) of HHCB in raw fish samples.

2.3. In vitro digestion

HHCB bioaccessibility was assessed in spiked raw hake using a static *in vitro* human digestion model described by Alves *et al* (2017). Briefly, the simulated gastro-intestinal digestion was performed with four digestion fluids: salivary, gastric, duodenal and bile. Each digestion fluid contained inorganic and organic components to simulate the three phases of the human digestion: oral, stomach and intestinal (Fig. S1). Each phase was analysed to quantify HHCB concentration, as explained below.

Then, raw homogenized hake was spiked at 10 µg g^{-1} in order to avoid concentrations under method quantification limit (MQL) in any digestion phase. Thus, two sets of spiked samples were prepared for practical reasons. 357 µL of standard HHCB solution at 168 mg L^{-1} in acetone were added to 6 g of homogenised hake samples (spike 1) and 476 µL of the same standard solution were added to 8 g of homogenised hake sample (spike 2). Both spiked samples (spike 1 and spike 2) were covered with acetone to facilitate the homogenous distribution of HHCB, then stirred several times and left in the fridge for two days to ensure equilibrium. A non-spiked hake sample (blank sample) was also analysed and its signal was subtracted from the corresponding signal of each spiked sample.

Several independent digestions were performed by using 1.5 g of spiked samples and stopped in each digestion phase. Thus, a first set of digestions was stopped in the oral phase and a second set was stopped in the stomach phase by using samples corresponding to spike 1 in triplicate. Then, a third set of complete digestions (oral, stomach and intestinal phases) was assessed by using samples from spike 2. In that case, 12 replicates were done to obtain enough amount of pellet, also named non-bioaccessible fraction (NBIO) (Fig. S1), to be analysed according to the analytical method used for solid samples, which is described in section 2.4.2. For each set, the digestion was stopped by placing the reaction

tubes on ice, followed by centrifugation at 2,750 g at 10°C during 10 min to separate the liquid and solid phases. The liquid phases were analysed according to the method described in section 2.4.3.

The percentage of HHCB in each phase was calculated according to equation 2.

$$\%HHCB = \frac{(m_a - m_{ba})x100}{m_s} \qquad (2)$$

Where m_a corresponds to the mass (ng) of HHCB present in the sample, m_{ba} is the mass (ng) of HHCB present in the blank of the corresponding sample and m_s is the mass (ng) of HHCB determined experimentally for spike 1 (samples for oral and stomach phases) or spike 2 (samples for intestinal phase). In the case of the non-bioaccessible fraction or pellet, twelve complete digestions were needed to obtain enough sample to be analysed. Then, concentration of HHCB was estimated in this fraction for one digestion.

To assess the *in vitro* digestion efficiency, total protein levels were determined using an FP-528 DSP LECO nitrogen analyser (LECO, St. Joseph, USA). Protein levels were measured in wet weight before digestion (BD), and in the bioaccessible (BIO) and non-bioaccessible (NBIO) fractions. The calibration standard curve was performed with EDTA following the methodology described by Saint-Denis & Goupy (2004).

Protein recovery (%) was defined as the following ratio:

((BIO+ NBIO) x 100)/(BD)

Where BIO + NBIO are the sum of protein levels detected in the bioaccessible (BIO) and non-bioaccessible (NBIO) fractions, and BD is the amount of protein detected in the sample before digestion.

Bioaccessible protein (%) was defined as the following ratio:

(BIO x 100)/(BD)

Where BIO corresponds to the protein levels detected in the bioaccessible fraction (BIO), and BD is the amount of protein detected in sample before digestion.

2.4. Methods of analysis

In order to analyse the different samples throughout the digestion process, as well as the cooked samples, two different extraction methods previously developed (Trabalón et al., 2015; Vallecillos et al., 2013) were applied and the extracts were analysed by gas chromatography ion trap tandem mass spectrometry (GC-IT-MS/MS). For solid samples, including raw and cooked fish samples and non-bioaccessible fractions (NBIO) or pellets, QuEChERS (Quick, Easy, Effective, Rugged and Safe) extraction method was applied (Trabalón et al., 2015). In contrast, for liquid samples, including oral, stomach and intestinal phase or bioaccessible fraction (BIO), the extraction was carried out by a head-space solid-phase microextraction (HS-SPME) method (Vallecillos et al., 2013).

2.4.1. Control of blanks

It is well known that working with musk fragrances require special precautions due to the widespread use of these compounds. In order to avoid contamination throughout the analytical procedure, all glass material was cleaned by using a sonication bath with ultrapure water for 20 minutes, followed by isopropanol for 30 minutes. In addition, the use of PCPs, such as creams, lotions, perfumes, deodorants, among others, were restricted for laboratory personnel (Chase et al., 2012; Liu et al., 2013). In order to assess the absence of contamination along the analytical procedure, blank analyses (analyses without sample) were performed in combination with sample analysis.

2.4.2. QuEChERS extraction

Solid samples were extracted by QuEChERS according to the method described by Trabalón *et al*. (Trabalón et al., 2015). Briefly, 0.5 g dry weight (d.w.) of freeze-dried solid sample, 10 mL of ultrapure water and 10 mL of acetonitrile (ACN) (HPLC grade, Prolabo) were mixed. Then, an extraction salt packet (Scharlab, Barcelona, Spain) was added according to

the Standard Method EN15662 (Lehotay et al., 2012) and the mixture was centrifuged. The ACN layer (supernatant) was removed and transferred to a 15 mL centrifuge tube containing 2 g of florisil (Sigma-Aldrich, Steinheim, Germany) for the dSPE (dispersive solid-phase extraction) clean-up. The tube was centrifuged and the supernatant was evaporated under a gentle stream of nitrogen to a final volume of, approximately, 1 mL. The internal standard (d_{15}-MX) was added and the extract was reconstituted to 2 mL with ethyl acetate. Extracts were filtered with a 0.22 mm PTFE (polytetrafluoroethylene) syringe filter (Scharlab) and 10 µL of the extracts were analysed by GC–IT-MS/MS.

2.4.3. HS-SPME extraction

Liquid samples were extracted by the HS-SPME method described by Vallecillos et *al*. (2013). The PDMS/DVB (polydimethylsiloxane/divinylbenzene) 65 µm fibre (Supelco, Bellefonte, PA, USA) was conditioned in line with the supplier's instructions, being inserted into the GC injector prior to the analysis. In resume, 10 mL of liquid sample was poured into a 20 mL vial and placed in a tray for SPME. When the temperature reached 60 °C, the vial was automatically transported, and the headspace was allowed to equilibrate with the sample at the extraction temperature during 5 min. The PDMS/DVB 65 µm fibre was then introduced through the septum and kept in the headspace of the vial for 30 min at 60 °C. During the extraction, the sample was magnetically stirred at 750 rpm. Desorption was conducted at 250 °C during 3 min in the GC injector. To prevent carry-over, the fibre was cleaned by heating to 250 ^{o}C during 10 min prior to every extraction, and a blank test (consisting in exposing the fibre to desorption conditions just after cleaning it) was performed to assess potential contaminations.

2.4.4 GC-IT-MS/MS analysis

The chromatographic analyses were performed according to the method described by Trabalón *et al* (2015) using a Varian ion trap GC-MS system (Varian, Walnut Creek, CA, USA), equipped with a 3800 gas chromatograph, a 4000 ion trap mass detector, a 1079 programmable vaporizing temperature injector and a CombiPAL autosampler (CTC,

Analytics, Zwigen, Switzerland). The chromatographic separation was carried out in a ZB-50 analytical column (50% phenyl-dimethylpolysiloxane, 30 m x 0.25 mm i.d.; 0.25 μm film thickness) from Phenomenex (Torrance, California, USA). The oven temperature was programmed as follows: 70 ^{o}C hold for 3.5 mins, raised at 50 ^{o}C min^{-1} to 200 ^{o}C, then 5 ^{o}C min^{-1} to 240 ^{o}C, and finally 20 ^{o}C min^{-1} to 290 ^{o}C and hold at 290 ^{o}C during 3.4 mins. The carrier gas employed was helium (99.999% purity, Abelló Linde, Barcelona, Spain) at a constant flow rate of 1 mL min^{-1}. The target compound and internal standard were eluted in 10 mins. The mass spectrometer was operated in the electron ionization (EI) mode (70 eV) and the system was controlled by Varian MS Workstation v.6.9 software. The transfer line, manifold and trap temperatures were 280 ^{o}C, 50 ^{o}C and 200 ^{o}C, respectively. For quantitative analysis of the target compound, tandem mass spectrometry (MS/MS) mode was applied. Table S1 summarises the retention time and the optimal MS parameters for HHCB and d_{15}-MX.

2.4.5. Quality control assurance

The criteria followed to ensure the correct identification of HHCB were: (1) the retention time should match that of the standard compound within ±1 s; (2) signal should be equal or higher than that corresponding to the limit of detection; (3) the signal ratios of product ions should match those corresponding to the standard compound within 15%.

For quantification purposes, matrix matched calibration curves were used because HHCB response is matrix dependent. d_{15}-MX was used as internal standard. In the case of digestion fluids, enzymes were not used to prepare solutions for calibration curves, in order to avoid HHCB degradation. Method detection limits were 1 ng g^{-1} (d.w.) and 0.25 μg L^{-1} for solid and liquid samples, respectively. Method quantification limits were 5 ng g^{-1} (d.w.) and 0.5 μg L^{-1} for solid and liquid samples, respectively.

2.5. Statistical analysis

All analysis were done in triplicate, which means that three independent aliquots were analysed. Differences between raw and cooked fish concentrations were analysed by *t*-test with a significance level set at 5%.

3. Results and discussion

3.1. Bioaccessibility study

The average concentrations of spiked and non spiked HHCB in hake samples, expressed as dry weight (d.w.), are shown in Table 1, as well as, the corresponding relative standard deviation (%RSD) and the average amount (ng) of HHCB in the sample. For non-spiked hake samples (blank samples), concentration was in accordance with previous results (Trabalón et al., 2015), and this value was subtracted to those obtained for spiked samples. Then, both spike 1 and spike 2 showed concentrations close to 10 μg g^{-1}, although spike 2 revealed lower precision. However, RSDs around 20% are acceptable values taking into account the complexity of the analytical method and the fish matrix (Vallecillos et al., 2015b).

Table 2 shows the average amount (ng) of HHCB in all analysed digestion phases of the *in vitro* digestion, the percentage (%) of HHCB in each digestion phase and their RSDs. In general, samples from hake revealed a high protein digestibility, indicating that the *in vitro* digestion protocol used in the present study successfully hydrolysed and realeased almost all proteins to be bioaccessible fraction (94.1 ± 2.6%). Although HHCB was not expected in any of the digestion fluid blanks, the salivary fluid contained small amount, thus being subtracted from the amount of oral phase. HHCB from hake became almost completely bioaccessible in the intestinal phase. In contrast, bioaccessible HHCB was very small in the

other phases. So far, no studies related to HHCB bioaccessibility have been reported in seafood. However, bioaccessibility studies from other EOCs, such as brominated flame retardants in seafood (Alves et al., 2017) like α-HBCD, was found to have high bioaccessibility in mackerel (89.6%) and plaice (62.8%). Bioaccessibility not only depends on the food matrix and composition of digestion fluids, but also on the physicochemical characteristics of the contaminants, such as the molecular weight and the octanol-water coefficient (Wang et al., 2010). Indeed, both α-HBCD and HHCB have a similar $logK_{ow}$ (5.6 and 5.9, respectively) and a high bioaccessibility. In addition, lipid content may also play a role in the uptake of contaminants at the intestinal level, as food with higher lipid content may decrease intestinal uptake of organic contaminants since lipids are difficult to digest and can retain lipophilic contaminants (Cunha et al., 2017). This is in agreement with the fact that hake is a low lipid content species, which could favor HHCB bioaccessibility.

3.2. Effect of cooking

The effect of steaming and grilling on HHCB concentration was investigated in non-spiked cod and mackerel samples. The present study was only focused on the fish because the liquid produced during the cooking processes, which can also contain HHCB, is not always ingested. Thus, it could be tested in further studies. The obtained results, expressed as ng g^{-1} in dry weight (d.w.), are summarised in Table 3 as well as the recoveries. Raw cod samples showed an average concentration of 76.9 ng g^{-1} (d.w.) HHCB, while raw mackerel samples showed a higher average value (103.7 ng g^{-1} d.w.). These values are in accordance with previous studies (Trabalón et al., 2015) and corroborate that the lipophilic properties of HHCB enables this compound to be at higher concentrations in high lipid content species.

Both steamed and grilled cooking processes promoted a significant reduction of HHCB levels in both species (see Table 3). Although grilling was expected to have higher influence on HHCB degradation, as it is a more aggressive cooking procedure, no significant differences were found between both cooking processes for both species (t-student, α=0.05).

On the other hand, the recoveries were affected by species, being around 50% for cod (representative of a low lipid content species) and around 30% for mackerel (representative of a high lipid content species). These results may be explained by the fact that cooking processes able to release fat also tend to reduce the levels of contaminant in cooked fish (Domingo, 2016). Regarding RSD values, although being acceptable, they were slightly higher for mackerel likely due to a higher variability in lipid content in fish matrix (Schlechtriem et al., 2012).

4. Conclusions

To the best of our knowledge, this is the first study dealing with the bioaccessibility of HHCB in fish samples, as well as the cooking effect on HHCB concentration. Preliminary results evidenced that the intestinal phase shows the highest bioaccessibility of HHCB, while oral and gastric phases revealed minor bioaccessibility. As a result, HHCB was near 100% bioaccessible at the end of the *in vitro* digestion procedure for raw hake samples. Moreover, steaming and grilling procedures induced a decrease in HHCB content (between 50% and 70% in cod and mackerel, respectively). Although both cooking processes did not show significant differences, higher reduction was generally observed in the highest lipid content species (mackerel) compared to the lowest lipid content species (cod).

In the future, more seafood species and cooking processes should be investigated to get a more realistic overview of HHCB exposure.

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Table 1. Average concentrations (n=3) of HHCB in spiked and non-spiked raw freeze-dried hake prior to *in vitro* digestion.

Sample	HHCB		
	ng g^{-1}(d.w.)	ng in sample	%RSD
Blank sample	22.2	11.1	10
Spike 1*	11899.4	5953.0	15
Spike 2*	13581.0	6795.4	18

(*) spiked at 10 μg g^{-1} (theoretical concentration)

Table 2. Average amount (ng) of HHCB in samples from *in vitro* digestion and digestion fluids' blank.

Phase	Sample	HHCB		
		ng in sample	%	%RSD
ORAL	Blank salivary fluid (n=1)	5.1		-
	oral phase (n=3)	37.6	0.6	15
STOMACH	Blank gastric fluid (n=1)	n.d.		-
	stomach phase (n=3)	103.0	1.7	9
INTESTINAL	Blank duodenal:biliar fluid (n=1)	n.d.		-
	intestinal phase (n=12)	9197.1	135	20
	Pellet (12 digestions pooled) (n=1)	1089.4		-
	Pellet (estimation for 1 digestion)	90.78	1.3	-

n.d.: not detected.
% percentage of HHCB (for calculations, see section 2.3)

Table 3. Concentrations in ng g $^{-1}$ (d.w.) and recoveries of HHCB in raw and cooked cod and mackerel samples.

	Cooking process	Concentration in sub-sample	%RSD	Average concentration	%Recovery
Cod	Raw	78.4	8	76.9	
		77.6	5		
		74.7	6		
	Steamed	47.3	12	41.6	54
		41.9	21		
		35.5	14		
	Grilled	39.7	7	37.5	49
		33.1	10		
		39.7	5		
Mackerel	Raw	98.7	16	103.7	
		107.9	21		
		104.4	17		
	Steamed	23.8	19	28.8	27
		31.6	18		
		30.9	17		
	Grilled	28.8	15	31.9	31
		36.7	15		
		30.1	20		

SUPLEMENTARY DATA

Table S1. Retention times and MS conditions.

Compound	Retention time (min)	Ions (m/z)	Product ions [a] (m/z)	Ionization Storage level (m/z)	CID Amplitude (V)	CID Storage level (m/z)	m/z range	Scan time (s/scan)
HHCB	8.99	243	171, **213,**	70	0.96	107	117-253	0.53
d_{15}-MX	9.40	295	170. **276**, 295	70	1.11	129	139-305	0.34

[a] Quantification ions (m/z) are shown in bold type.

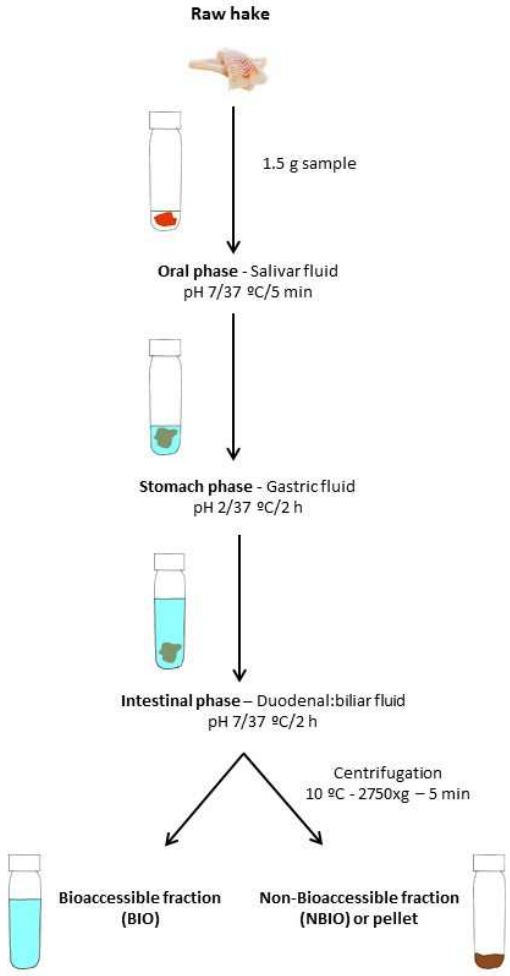

Figure S1. *In vitro* digestion scheme used to assess HHCB bioaccessibility.

Highlights

- An *in vitro* digestion protocol was applied.
- High galaxolide bioaccessibility in fish.
- Intestinal phase shows the highest bioaccessibility.
- Steaming and grilling reduces similarly galaxolide content.
- High lipid content species more affected by cooking process.