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Nutrient and contaminant exposure from smoked European anchovy (*Engraulis encrasicolus*): Implications for children's health in Ghana

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ABSTRACT

Inadequate nutrient intakes are frequent among young children in low- and middle-income countries, causing prevalent micronutrient deficiencies. In Ghana, small smoked fish are the most frequently consumed animal source foods, and both whole fish and different tissues of fish are commonly used in complementary foods. However, the risks and benefits associated with consumption of different tissues of smoked fish has not been explored. Samples of smoked European anchovy (Engraulis encrasicolus) were collected from five regions in Ghana and divided into subsamples of different tissues: whole fish, heads and skin, and samples without heads and skin. The different tissues were subsequently analyzed for selected nutrients (vitamins, minerals, trace elements and fatty acids), heavy metals and polycyclic aromatic hydrocarbons. A scenario referencing young children in Ghana (aged 6-23 months) was developed to assess the risks and benefits associated with daily consumption of different tissues of smoked European anchovy. We found that all tissues had the potential to substantially contribute to the recommended nutrient intakes of vitamins, minerals and essential fatty acids important for young child development. Samples of whole fish and of heads and skin contained levels of cadmium which exceeded the tolerable weekly intake greatly in the current scenario. All tissues contained elevated concentrations of BaP and PAH4 which exceeded the margin of exposure in the scenario, entailing potential consumer risk. A thorough assessment of dietary intakes of smoked fish products and refined risk-benefit assessments are therefore imperative to secure safe utilization of smoked fish in Ghana.

1. Introduction

The global burden of malnutrition is increasing along with the need to access nutritious and sustainable foods (FAO et al., 2020). Despite widespread recognition of its nutritional value, use of fish is largely unrecognized as a potential food-based approach to alleviate malnutrition (Hasselberg, Aakre, et al., 2020; Hicks et al., 2019). Fish offers a unique contribution to food and nutrition security, specifically in many coastal low- and middle-income countries (LMICs) (FAO, 2018), where small pelagic fish species serve as the primary source of animal protein, omega-3 fatty acids, vitamins and minerals (Béné et al., 2015; Kawarazuka & Béné, 2011). In Ghana, fish constitutes 50–80% of total animal-source protein originating from diverse and bountiful aquatic resources along the continental coastline and inland waterbodies (FAO, 2016; Sumberg et al., 2016).

To prolong shelf life and enable transportation without cooling, small fish are commonly preserved by smoking in traditional open flame chorkor and oil drum kilns in Ghana, which generates high levels of polycyclic aromatic hydrocarbons (PAHs) (Essumang et al. 2012, 2013; Hasselberg, Wessels, et al., 2020). PAHs are a group of ubiquitous compounds formed during incomplete combustion of organic matter and are considered a major concern for human health due to their different carcinogenic and mutagenic properties (EFSA, 2008; Phillips, 1999; Singh et al., 2016). In addition, elevated levels of toxic heavy metals may be present in processed fish, since anthropogenic activities including e-waste recycling is increasing in many LMICs, including Ghana (Gupta et al., 2019; Kumar et al., 2019; Yabe et al., 2010). For instance, Agbogbloshie in Accra, the capital of Ghana, is home to one of the largest e-waste recycling cites in the world, where haphazard waste management release Pb, Cd, Hg, As and other metalloids into the

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surroundings (Itai et al., 2014; Kyere et al., 2017; WHO, 2015).

Infants and toddlers require essential nutrients for growth and development and are particularly sensitive to PAH and heavy metal exposure due to their limited ability of detoxification compared with adults (Ginsberg et al., 2004; Scheuplein et al., 2002). There is global consensus that suboptimal nutritional status during the first 1000 days of a child's life, from conception through age 2 years, combined with toxicant exposure have severe implications for children's ability to reach their developmental potential (Black et al., 2017; Grantham-McGregor et al., 2007; Landrigan & Goldman, 2011; Stewart et al., 2013). In Ghana and other LMICs, inadequate feeding during the transition from exclusive breastfeeding to complementary feeding remains one of the main causes of malnutrition in children under 5 years (Keats et al., 2021; UNICEF, 2019). Traditional Ghanaian complementary foods are predominantly based on micronutrient-poor staple foods including fermented maize porridge (*koko*), millet or ready-to-use cereal and legume blends containing high levels of phytate (Amangloh et al., 2011; Lartey et al., 1999). Adding powdered whole small fish to complementary foods is also common practice in Ghana (Amangloh et al., 2011; Lartey et al., 1999) and including heads, skin or other cutoffs has been proposed as a sustainable way to utilize fish and increase micronutrient intakes (Abbey et al., 2017).

Several studies and reports have disseminated the risks and benefits associated with fish consumption, however, the compositional data used is primarily of unprocessed fish from Europe, US and Japan (Cohen et al., 2005; FAO/WHO, 2010; Hellberg et al., 2012; Ho et al., 2021; Nøstbakken et al., 2021). Thus, the subsequent exposure-scenarios are not compliant with a Ghanaian setting, where the majority of consumed fish is of local origin and in processed form. There is a dearth of studies reporting the risks and benefits of consuming locally processed fish in Ghana and other LMICs and this study provides novel data on nutrient and contaminant levels in tissues of smoked European anchovy from Ghana.

The aims of the present study are to determine the distribution of selected nutrients and contaminants in different tissues of smoked European Anchovy and to perform an initial risk-benefit analysis in a scenario referencing young Ghanaian children. Risk assessment of food is of great importance for public health, and assessing the concentration of key nutrients, heavy metals and PAH-contamination in tissues of one of the most frequently consumed species in Ghana provides important data for local nutritionists, toxicologists and policy makers.

2. Materials and methods

2.1. Sampling protocol

Samples of smoked *Engraulis encrasicolus* (European anchovy) were collected from open fish markets in five regions throughout Ghana in November 2018, namely; Accra Agbogbloshie and Adabraka Market in the coastal Greater Accra region, Kumasi Central Market in the Ashanti region, Techiman Market in the Brong-Ahafo region (now Bono East), Tamale Market in the Northern region and Bolgatanga Market in the Upper East region. Calculation of sample size and development of the sampling protocol was based on Greenfield and Southgate's requirements for retail sampling as described in Hasselberg, Wessels, et al. (2020).

2.1.1. Sampling procedure

Five batches of approximately 300 g smoked European anchovy (>50 fish) were sampled from the above listed fish markets (n = 5) between 2nd and November 24, 2018. To cover a larger area of the fish markets, the selected fish species were requested at every third market stall until five batches were obtained. Each batch was packed in zip-lock polyethylene bags at the collection site and transported in insulated polystyrene boxes during transit.

2.1.2. Sample preparation

Key persons[1] in Ghana were consulted to determine the optimal tissue analyses to assess the potential benefits and health hazards associated with consumption of smoked European anchovy. Hence, the 25 batches of smoked European anchovy were pooled into 5 composite samples and subsequently divided into three subsamples: **1)** whole fish (WF), **2)** heads and skin (HS) and **3)** without heads and skin (WHS). In accordance with local practices, the subsamples were beheaded and deskinned by hand at the CSIR Food Research Institute in Accra, Ghana. This resulted in a total of n = 15 technical replicates, representing single analytical values of three different tissues per region.

2.2. Analytical methods

Prior to analyses, the different tissues were homogenized in a food processor (Braun 3210, Neu-Isenburg, Germany) and subsamples of the homogenate were divided into tubes (Thermo Scientific Nunc A/S, Roskilde, Denmark) and stored at −20 °C. Analytical samples for vitamin analyses were stored at −80 °C. Analytical samples for crude fat-, protein-, element- and PAH-analysis were freeze dried in accordance with the AOAC 930.15 method described by Thiex (2008), while analyses of vitamins and fatty acids were performed using wet sample material. Detailed descriptions of the analytical methods are presented in Moxness Reksten, Bøkevoll, et al. (2020).

2.2.1. Determination of crude protein and fat, fatty acids, vitamins, elements and PAHs

The crude protein content was determined by the Dumas method (AOAC, 1995) using a Leco FP 628 nitrogen analyzer (Leco Corporation, Saint Joseph, MI, USA) and N × 6.25 as nitrogen to protein conversion factor. The composition of fatty acids was determined by extracting lipids from the samples according to Folch et al. (1957), and subsequently analyzing the fatty acid composition of total lipids by gas chromatography (GC) coupled with a flame ionization detector according to Lie and Lambertsen (1991) with modifications described by Fauske et al. (2018), using 19:0 methyl ester as an internal standard. Vitamin A_1 (sum of all *trans*-retinol and 13-, 11-, 9 cis retinol) was determined using high-performance liquid chromatography (HPLC) and a Photo Diode Array detector (HPLC 1260 system Agilent Technologies, PDA, Santa Clara, CA, USA) according to ECS (2000) and retinol content was calculated using an external standard curve. Vitamin D_3 content was determined by HPLC using an UV detector (HPLC LaChrom Merck HITACHI system, Tokyo, Japan) as described by ECS (2009) and the D_3 concentration was calculated using vitamin D_2 as an internal standard.

Quantification of Cobalamin (vitamin B_{12}) was completed by adding a microorganism (Lactobacillus delbruecki -ATCC 4797) to the sample material followed by turbidimetric reading (Optical Density, OD, v/575 nm) according to Angyal (1996). Concentration of elements Fe, Se, Zn, I, Ca and As was determined by inductively coupled plasma-mass spectrometry (ICP-MS) (iCapQ ICP-MS, ThermoFisher Scientific, Waltham, MA, USA) equipped with an auto-sampler (FAST SC-4Q DX, Elemental Scientific, Omaha, NE, USA) after wet digestion in a microwave oven (UltraWave, Milestone, Sorisole, Italy), as described by Julshamn et al. (2007). For I, specifically, tetramethylammonium hydroxide was used for extraction. An external standard curve was used to determine the concentration of the elements in addition to different internal standards, specified in detail in Moxness Reksten, Bøkevoll, et al. (2020). Methylmercury (MeHg) content was determined by GC-ICP-MS using an Agilent (Santa Clara, CA, USA) 6890 N gas chromatograph coupled with an Agilent 7500a ICP-MS instrument according to Valdersnes et al. (2012) and quantified using an isotope dilution. Inorganic As (iAs) was determined using an Agilent 1260 Infinity II Series BIO Inert HPLC with an

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autosampler (Santa Clara, CA, USA) coupled with an Agilent 7900 ICP-MS instrument as described by Sloth et al. (2005) and quantified using external calibration. Concentration of the 16 EU priority PAHs (listed in Table A1) was determined by GC-MS/MS (GC: 7890A GC System; MS: 7000B Triple Quad) coupled with an autosampler (7693 Agilent Autosampler) as described by Moxness Reksten, Bøkevoll, et al. (2020). A four-point calibration curve (15 + 1 EU PAH Cocktail, Chiron) was used for quantification.

2.2.2. Quality control and quality assurance

All laboratory analyses were performed at the Institute of Marine Research (IMR), Norway, using validated and accredited methods according to ISO 17025:2005. This does not include the analytical method for Fe, which is validated but not accredited. As described in Moxness Reksten et al., 2020 IMR-laboratories regularly participate in national and international proficiency tests to assess the accuracy, precision and measurement uncertainty of each analytical method. The quality control includes annual analyses of certified reference materials, whereas internal reference materials are included in each sample run for quality control. An overview of the validated measurement ranges and measurement uncertainties (%) for each analytical method is presented in Table A1, adapted from Moxness Reksten et al., 2020.

2.3. Scenario design

A scenario was developed to evaluate the benefits and risks associated with consumption of different tissues of smoked European anchovy from Ghana. Infants and toddlers aged 6–23 months were chosen as the target population since inadequate feeding practices and micronutrient deficiencies are common in this population group (GSS, 2014; Agbadi et al., 2017). Data on the use of fish in complementary foods in Ghana is limited, and the amount (e.g. portion size) of 17 g used to calculate different points of departure was derived from a set of publications (Amangloh et al., 2011; Bogard, Hother, et al., 2015; Lartey et al., 1999). A sex-neutral weight-for-age average (6–23 months) of 10 kg was derived from WHO's Child Growth Standards (WHO, 2006). Analytical data on whole European anchovy from Hasselberg, Wessels, et al. (2020), as highlighted in Table A3, was included to enable a risk-benefit assessment of the three different tissues.

2.3.1. Recommended nutrient intakes

To assess the nutritive potential of different tissues of smoked European anchovy, a single target for recommended nutrient intakes (RNI) was assigned for each nutrient. A mean value was calculated from FAO/WHO recommendations (FAO/WHO, 2004) for the selected age group (6–23 months), as previously described by Bogard, Thilsted, et al. (2015). The RNI-targets are presented in Table 1 and the calculation of each RNI-target is presented in Table A2. The contribution of a 17 g portion to the RNI-targets was subsequently calculated for each tissue. Absorption rates for Zn and Fe were set to 30% and 10%, respectively, due to the high levels of phytate and low presence of animal source foods (ASFs) in a typical Ghanaian diet (Colecraft et al., 2006; Nti, 2008). FAO/WHO recommendations were not available for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) fatty acids and the European Food Safety Authority's (EFSA, 2012) recommended dietary intake (RDI) was used.

2.3.2. Upper tolerable intake levels

Upper tolerable intake levels (UL) were obtained from multiple sources, principally selecting the most conservative estimation of ULs. Accordingly, 50% of the ULs were obtained from EFSA (2018) and the remainder from the US National institute of Health (Institute of Medicine, 2001; Institute of Medicine, 2011) and The Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2019a). ULs were not available for Fe, and the provisional maximum tolerable daily intake (PMTDI) was therefore used (JECFA, 2019a) (Table 1). The calculation of each UL-target is presented in Table A2.

2.3.3. Provisional tolerable weekly intake and tolerable weekly intake

The provisional tolerable weekly intake (PTWI) of 1.6 µg/kg/b.w./week for MeHg from JECFA (2019b), and tolerable weekly intake (TWI) of 2.5 µg/kg/b.w./week for Cd from EFSA (2011), were used to calculate the contribution of daily 17 g servings to the PTWI/TWI (%) using mean analytical values for MeHg and Cd in the different tissues of European anchovy.

2.3.4. Margin of exposure

The daily dietary exposure to selected PAH congeners including PAH4 (sum of benzo[*a*]pyrene (BaP), chrysene, benz[*a*]anthracene and benzo[*b*]fluoranthene) and BaP in addition to Pb was calculated using the formula (Eq. (1)).

$$Daily\ dietary\ exposure = \frac{average\ concentration\ (PAH4,\ BaP\ or\ Pb)\left(\frac{ng}{g}\right) x\ daily\ consumption\ \left(\frac{g}{day}\right)}{body\ weight\ (kg)} \tag{1}$$

Margins of exposure (MOEs) were calculated using EFSA BMDL $_{10}$ values for PAH4 (0.34 mg/kg/b.w./day) and BaP (0.07 mg/kg/b.w./day) (**Eq. (2)**) (EFSA, 2008). For Pb, the MOE for children 1–3 years was calculated using a $BMDL_{01}$ dietary intake value of 0.50 µg/kg/b.w./day, which has been associated with intellectual deficits in children (**Eq. (2)**) (EFSA, 2010a).

Table 1
Calculated daily targets for selected nutrients for infants and toddlers (6–23 months), and data sources.

Nutrient	Unit[a]	Target[b]	Source
Calcium	RNI	450 mg	FAO/WHO (2004)
	UL	2000 mg	Institute of Medicine (2011)
Zinc	RNI	270.5 µg/kg/bw/day	FAO/WHO (2004)
	UL	6 mg	Institute of Medicine (2001)
Iodine	RNI	90 µg	FAO/WHO (2004)
	UL	200 µg	EFSA (2018)
Iron	RNI	7.5 mg	FAO/WHO (2004)
	PMTDI	0.8 mg/kg bw/day	JECFA (2019a)
Selenium	RNI	13.5 µg	FAO/WHO (2004)
	UL	60 µg	EFSA (2018)
Vit D_3	RNI	5 µg	FAO/WHO (2004)
	UL	30 µg	EFSA (2018)
Vit B_{12}	RNI	0.8 µg	FAO/WHO (2004)
EPA + DHA	RDI	100 mg	EFSA (2012)

[a] Original unit for the different nutrients.
[b] Calculated from multiple values presented in Table A2.

$$\text{Margin of exposure} = \frac{\text{BMDL}_{10/01}\left(\frac{ng}{kg\ b.w\ day}\right)}{\textit{Estimated daily exposure}\left(\frac{ng}{kg\ b.w\ day}\right)} \quad (2)$$

2.4. Data expression and statistical analyses

Analytical values of PAHs below the limit of quantification (LOQ) are presented as the upper bound (UB) using the respective LOQ-value for calculations. Statistical analyses were performed using GraphPad Prism version 9.0.2 (GraphPad Software LLC) and a significance level of $\alpha = 0.05$ was used for all tests. Differences between the tissues were determined using a Kruskal-Wallis test followed by a Dunn's post hoc test for pairwise multiple comparisons.

3. Results and discussion

3.1. Nutrients

All tissues analyzed had the potential to substantially contribute to the RNIs of selected vitamins, minerals and essential fatty acids (Fig. 1A). The proximate composition, selected vitamins and minerals in the different tissues of smoked European anchovy from each sampling site are reported in Table A3. Samples containing HS had the highest concentrations of all minerals, vitamins and FAs, with a 17 g portion contributing >50% to the RNI-targets for all selected nutrients except I, followed by >40% for WF and >25% for WHS- samples. Portions (17 g) of the different tissues all contained concentrations below the UL-targets for selected nutrients (Fig. 1B).

3.1.1. Potential contribution to recommended nutrient intakes and upper tolerable intake levels

Vitamin B_{12} content in all tissues was sufficient to cover the RNI-target (>230%, Fig. 1A). The highest content of B_{12} was determined in samples containing HS (Fig. 1A), which is in line with earlier studies identifying dark fish muscle as a target organ for B_{12} deposition (Nishioka et al., 2007; Watanabe, 2007). Yet, while dark muscle is primarily located directly under the skin of pelagic fish (Albrecht-Ruiz & Salas-Maldonado, 2015), the exact distribution of dark muscle in the different tissue samples remains uncertain. Vitamin B_{12} is only present in ASFs (Watanabe, 2007) and deficiency is usually uncommon among non-vegetarians. However, given the low consumption rates of ASFs in many low-income households, B_{12} deficiency is likely present among Ghanaian children (Wegmüller et al., 2020).

Oily fish is considered the principal dietary source of vitamin D for humans (Lock et al., 2010) and a 17 g portion of all tissues of smoked European anchovy could provide substantially to the RNI-target, ranging from 44% in WF, to 46% in WHS-samples and 57% in HS-samples (Fig. 1A). Although some vitamin D deposition has been registered in gills and skin of Atlantic salmon (Horvli et al., 2002), detection of the highest levels of vitamin D in HS-samples was unexpected but may be attributed to higher fat-content (Table A3).

The omega-3 fatty acids EPA and DHA are essential for optimal nervous tissue growth and function during infant- and toddlerhood (Innis, 2008; Luchtman & Song, 2013), but a steady decrease in DHA-intake from the onset of complementary feeding has been observed in many LMICs (Huffman et al., 2011). Our results demonstrate that all tissues of European anchovy were good sources for EPA and DHA, with an 17g portion of HS contributing 340% of the RNI and both WF and WHS-samples contributing 306% (Fig. 1A).

Considering the high proportion of bones and cartilage present in HS samples, the concentration of Ca was, as expected, significantly higher in samples of HS than WHS ($p = .0009$, Fig. 1A). However, all tissues were good sources of dietary Ca (>49% RNI-target) and well within the UL of 450 mg (Fig. 1B). According to WHO, non-breastfed children (6–23 months) should receive milk or dairy products 2–3 times daily to ensure adequate Ca intake (WHO, 2007), yet only 11% of Ghanaian children received the minimum number of servings (GSS, 2014), thus emphasizing the importance of alternate food sources.

Fish are recognized as an excellent dietary source of Se (Fairweather-Tait et al., 2010), which plays a critical role in reproduction, DNA synthesis and prevention of oxidative damage (Rayman, 2012). Our data demonstrated that all tissues contained substantial amounts of Se, ranging from 254% coverage of the RNI-target from HS-samples to significantly lower coverage (164%, $p = .0115$) from samples WHS (Fig. 1A).

Marine fish are known as the richest natural food sources of I (Haldimann et al., 2005), which plays a foundational role in neurodevelopment during the first 1000 days of life (Schwarzenberg & Georgieff, 2018; Velasco et al., 2018). The current study documented that different tissues of smoked European anchovy are good sources of dietary I, with a portion of HS-samples contributing significantly more to the RNI-target (46%) compared with WHS-samples (23%) ($p = .0041$, Fig. 1A). Although salt iodization has been implemented as the key global strategy to eliminate I deficiency, including in Ghana, a previous dietary intervention in the Upper Eastern region of Ghana demonstrated that goitrous children gained significantly improved I status following two weeks on a diet enriched with marine fish (Atlantic cod), concluding that consumption of marine fish could be a promising food-based strategy to secure adequate I intake (Maage et al., 2008).

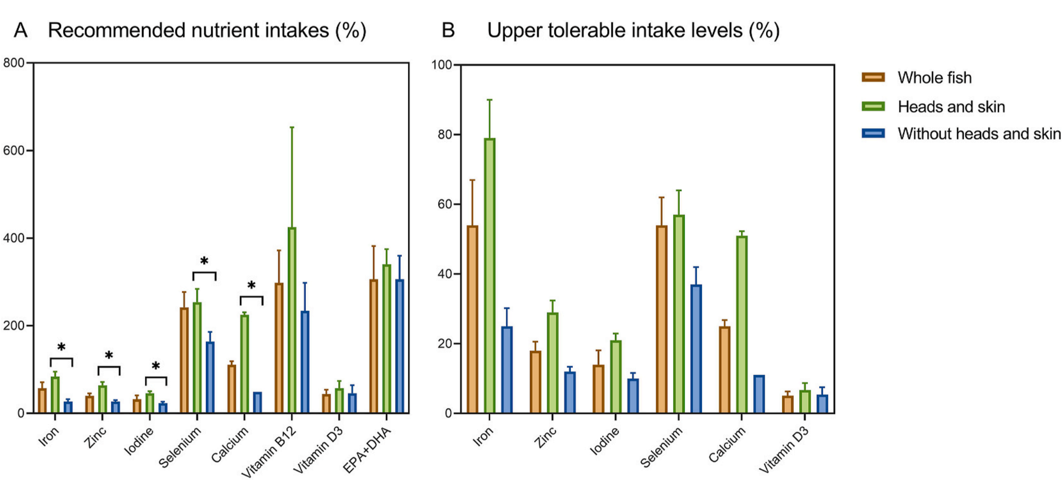

Fig. 1. Mean contribution (%) of an estimated portion (17 g) of different tissues of European anchovy to the **(A)** RNI-targets and **(B)** UL-targets of selected nutrients for infants and toddlers. Data represents mean ± SD (%) (n = 5). Significant differences (p < 0 0.05) between the groups are marked with *.

Zn deficiency is assumed to be prevalent in Sub-Sahara (Hess, 2017) and is associated with increased infectious morbidity and reduced linear growth (Black et al., 2013). Concurrent with previous reports (Kawarazuka & Béné, 2011; Bogard, Thilsted, et al., 2015; Ahern et al., 2020), we confirmed that small fish eaten whole are good sources of dietary Zn. In particular, samples of HS contained significantly high concentrations of Zn (p = .0016), providing 64% to the RNI-target from a 17 g portion (Fig. 1A).

Anemia remains one of the most persistent health burdens in Ghana affecting 35–66% of children under five years (GSS, 2014; University of Ghana et al., 2017) and is primarily caused by a scarcity of dietary Fe combined with infectious disease (Wegmüller et al., 2020). In line with Roos et al. (2007), samples containing HS could provide a significantly higher proportion of the RNI-target (84%) compared with samples WHS (27% RNI-target, $P = .0012$) (Fig. 1A). Including food commodities rich in bioavailable heme Fe such as fish in the diet is one of WHO's key recommendations for adequate complementary feeding (WHO, 2007) and inclusion of oily fish in phytate rich meals has also been known to counteract the inhibitory effect of phytates and increase absorption non-heme Fe (Navas-Carretero et al., 2008). The low bioavailability of Fe from Ghanaian complementary foods (Amangloh et al., 2011; Lartey et al., 1999) was accounted for by setting the bioavailability at 10%, which yielded a narrow limit between the calculated RNI-target (7.5 mg) and provisional maximum tolerable daily intake (PMTDI, 0.8 mg/kg/bw/day). Notably, the PMTDI refers to total dietary Fe and with a relatively modest portion (17 g) of samples containing HS solely contributing 79% of the UL (Fig. 1B), total dietary Fe may surpass the UL.

Consuming ASFs during early childhood is a key strategy for breaking the cycle of malnutrition (Iannotti, 2018). Smoked and dried small fish are particularly potent ASFs in this context since the reduced water content from processing increases the nutrient-density of several key nutrients (Byrd et al., 2021; Hasselberg, Wessels, et al., 2020). Our study confirmed that small amounts of different tissues of smoked European anchovy have the potential to ameliorate inadequate nutrient intakes among young children in Ghana. This may also be plausible in the context of food security, given that processed small fish are the most available and accessible ASFs throughout Ghana (Aheto et al., 2012) and

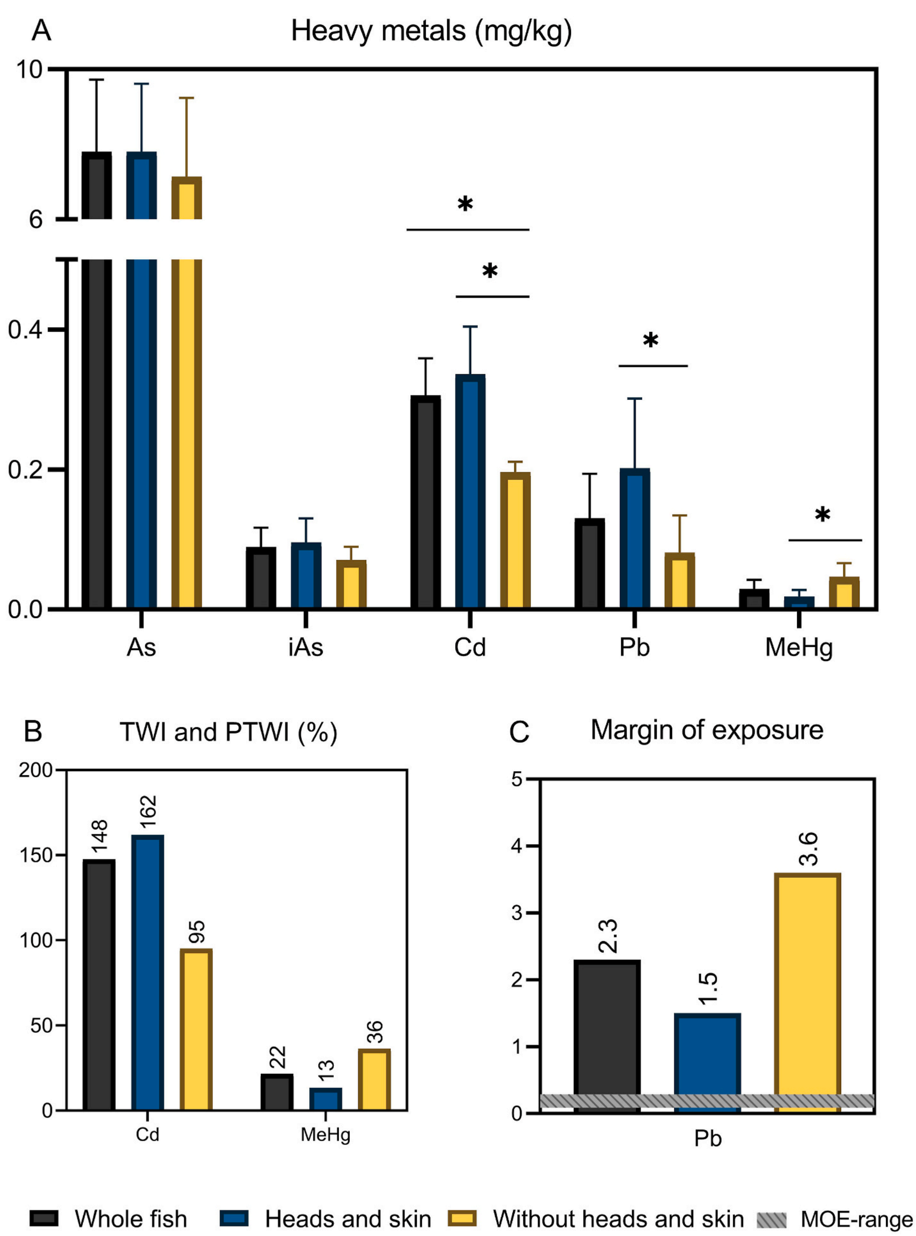

Fig. 2. (A) Mean concentration of heavy metals arsenic, inorganic arsenic, cadmium, lead and methylmercury (mg/kg), **(B)** mean contribution to TWI/PTWI for cadmium and methylmercury from weekly consumption of different anatomical sections of European anchovy, **(C)** Margin of exposure (MOE) for lead in different anatomical sections of European anchovy, with the shaded area representing the EFSA MOE-range (0.09–0.29) for high consumers of Pb-rich foods in the EU (1-3-years of age). Data represents mean/± SD (n = 5). Significant differences (p < 0 0.05) between the groups are marked with *.

utilization is deeply rooted in Ghanaian food culture (Colecraft et al., 2006).

3.2. Contaminants

Determination of MeHg, As, iAs, Pb and Cd in different tissues of European Anchovy displayed significant differences for multiple heavy metals (Fig. 2A/Table A3). HS-samples contained the highest concentrations of As, iAs and significantly higher levels of Cd and Pb compared with WHS-samples, while WHS-samples contained significantly higher concentrations of MeHg than HS-samples (Fig. 2A). Samples of WF contained intermediate concentrations of the majority of the metals analyzed, but a significantly higher concentration of Cd compared with WHS-samples (Fig. 2A). Analysis of the 15 + 1 EU priority PAHs determined that HS-samples contained the highest mean concentration of PAH 15 + 1, PAH4 and BaP, followed by WF, and the lowest concentrations were determined in WHS-samples (Table A4). Calculation of MOEs for BaP and PAH4 illustrated that all fish tissues contained severely elevated levels of PAHs (Fig. 3) and displayed similar PAH-profiles (Fig. 4).

3.2.1. Analytical levels and potential exposure to heavy metals

MeHg, the most toxic form of Hg, is known to bio magnify and bioaccumulate in the marine food chain (Clarkson & Magos, 2006) and may cause acute and chronic health effects at low exposure levels (Bose-O'Reilly et al., 2010). In the samples of smoked European anchovy, the concentration of MeHg was significantly higher in WHS-samples (0.018 mg/kg) compared with HS-samples (P = .0337, Fig. 2A). This resonates with previous analyses of different tissues of

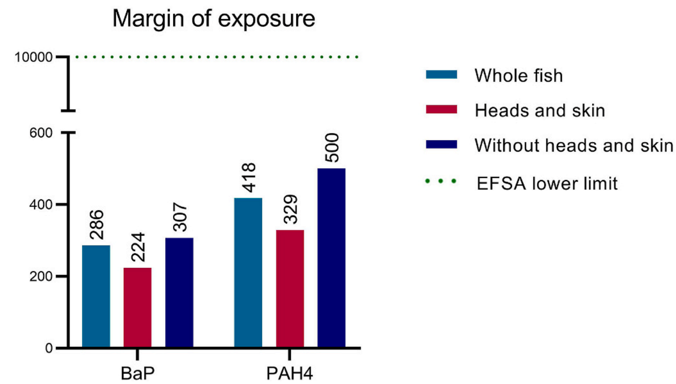

Fig. 4. Margin of exposure (MOE) for BaP and PAH4 in different tissues of European anchovy, with the dotted line marking the EFSA MOE-threshold. Data represent means (n = 5).

fish, where fish muscle has been identified as the main target for MeHg-accumulation (Amlund et al., 2007). Speciation of MeHg enabled calculation of PTWIs which illustrated that all sampled tissues contributed <40% of the PTWI (13–36%, Fig. 2B), indicating a relatively low MeHg exposure from smoked European anchovy in the current scenario.

The developing brain is vulnerable to the detrimental health effects associated with Pb-exposure, which is a recurrent issue in Ghana and other LMICs (Needleman, 2004; Flora et al., 2012; WHO, 2015). Pelagic fish species in their raw form are known to contain low levels of Pb in muscle (Vieira et al., 2011; Moxness Reksten, Bøkevoll, et al., 2020) and WF (Institute of Marine Research, 2018), however, elevated levels of Pb

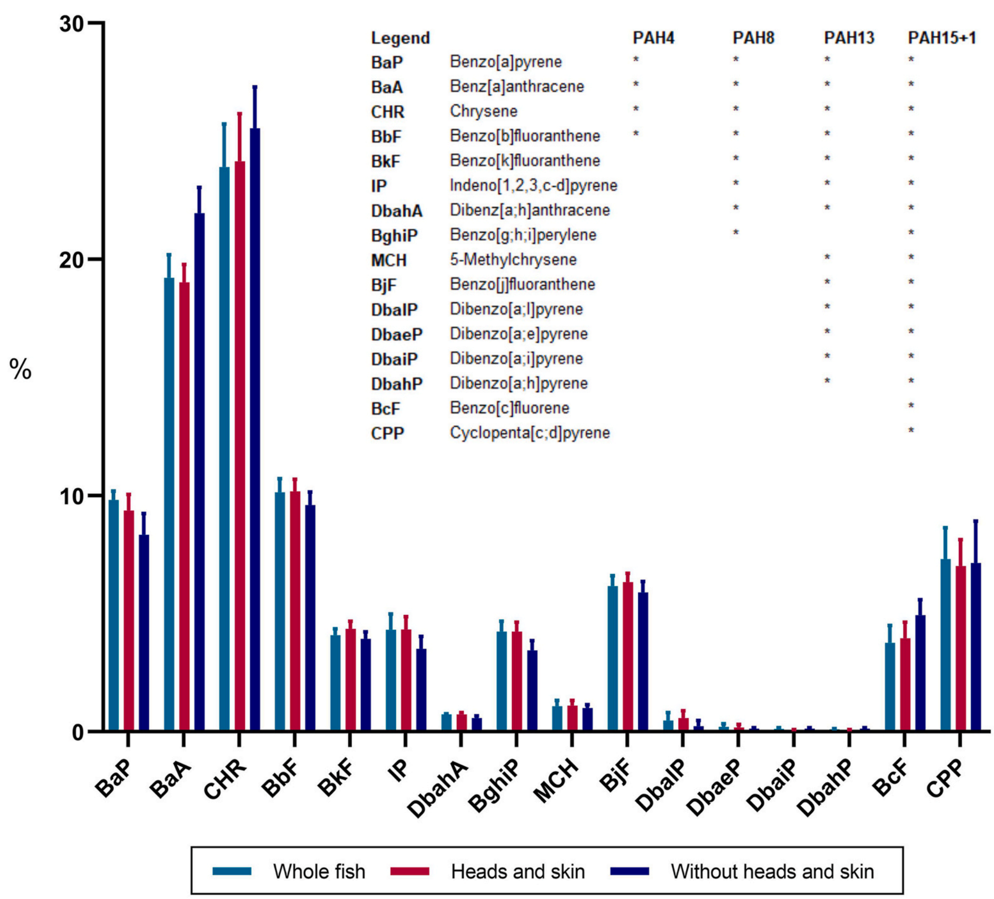

Fig. 3. Relative contribution of PAH congeners to PAH15 + 1 (%) in different anatomical sections of European anchovy. Data represents mean ± SD (%) (n = 5).

(1.3 mg/kg) have been detected in dried small freshwater species acquired in Accra (Hasselberg, Wessels, et al., 2020). Correspondingly, the highest values of Pb in the current study were determined in tissue samples from Accra, with HS-samples from this specific area (0.350 mg/kg, Table A3) exceeding the EU-limit for Pb in fish muscle (0.3 mg/kg) (European Commission, 2006). However, the location-specific data represent a single analytical value per tissue and can only serve as a possible indicator of Pb-pollution in Accra. Overall, the concentration of Pb was significantly higher in samples of HS compared with WHS-samples (Fig. 2A, $p = .0388$), but neither exceeded the regulatory limits of 0.3 mg/kg. By calculating the MOE, it was determined that all samples exceeded the MOE-range of 0.09–0.29 based on high consumers (1–3 years of age) of Pb-rich foods in the EU (Fig. 2C). It has been determined that the risk is likely to be low For MOEs greater than 1, which was exceeded for all tissue samples in the current study (Fig. 2C) (EFSA, 2010a). However, this is assuming a negligible exposure from air and, soil and dust, which is not the case for Ghanaian children living in urban settings or close to environmental hotspots such as the Agbogbloshie e-waste recycling site. The Agbogbloshie fish market where the samples of smoked European anchovy from Accra were retrieved is located adjacent to the Agbogbloshie e-waste recycling site, a known source of Pb pollution (Itai et al., 2014; Kyere et al., 2017).

The concentration of As ranged from 7.1 mg/kg in WHS-samples to 7.8 mg/kg in both WF and samples consisting of HS, displaying no significant differences between the tissues (Fig. 2A). Fish and seafood are known as primary contributors to dietary As-intake (Llobet et al., 2003); however, the toxicity of As compounds depends on the chemical form of the element (Julshamn et al., 2012; Mania et al., 2015). Speciation of both total-and iAs demonstrated that >98% of As present in the samples was organic As (e.g arsenobetaine), which is known to not cause any detrimental health effects. This corresponds to previously reported As/iAs ratios in raw fish fillet, where >95% was organic As (Mania et al., 2015), thus indicating that the ratio remains stable throughout the smoking process and in different types of tissue. However, the concentration of iAs was markedly higher than previously reported findings in fillets of fresh fish, including high trophic species such as saithe (0.015 mg/kg, Julshamn et al., 2012) and tuna (0.008 mg/kg, Sloth et al., 2005), and should be monitored in future food safety assessments of processed fish.

The samples consisting of HS contained significantly higher concentrations of Cd (0.34 mg/kg) compared with samples WHS (0.20 mg/kg) ($P = .0112$), and WF (0.31 mg/kg) had significantly higher concentrations of Cd compared with WHS-samples ($P = .0405$) (Fig. 2A). This is consistent with previous reports of greater Cd accumulation in the liver (Boalt et al., 2014), gills (Chowdhury et al., 2004) and WF (Julshamn et al., 2004) compared with fish muscle. The average concentration of Cd in WF and WHS-samples exceeded the EU regulation for muscle meat of *Engraulis* spp of 0.25 mg/kg (European Commision 2006), which prompted further calculations of Cd-exposure. Calculation of TWI demonstrated that daily consumption of a portion (17 g) for a young child exceeded the Cd TWI substantially for WF (148%) and HS-samples (162%) (Fig. 2B). Considering the diverse toxic effects associated with Cd exposure, including nephrotoxicity (Rani et al., 2014) and impaired neuropsychological development in children (Rodríguez-Barranco et al., 2014), consumption of whole and HS smoked European anchovy will entail potential consumer risk in the current scenario. The elevated levels of Cd and other metals in the present study are likely attributable to the cumulative effect fish smoking has on metal-and mineral retention (Hasselberg, Wessels, et al., 2020), although the potential influence of elevated Cd-levels determined in soil and sediments in coastal Accra and Agbogbloshie should not be dismissed (Chama et al., 2014; Itai et al., 2014). Elevated blood and urinary concentrations of Cd have been documented in children living in polluted areas similar to Agbogbloshie (de Burbure et al., 2006), which calls for further identification and mitigation of anthropogenic Cd pollution in Ghana.

3.2.2. Distribution of polycyclic aromatic hydrocarbons and potential consumer risk

There are currently no legal limits available from the Ghana Standards Authority for PAHs in foodstuffs and the EU maximum limits of 2 μg/kg BaP and 12 μg/kg PAH4 in muscle meat of smoked fish and smoked fishery products are therefore used as a reference (European Commission, 2011). The highest mean concentration of PAHs occurred in the composite samples of HS, which contained 45-and 51-fold the EU maximum limits for BaP (90 ± 25 μg/kg) and PAH4 (608 ± 460 μg/kg) (Table A4), respectively. Levels of BaP and PAH4 detected in WHS-samples were lower at 51 ± 15 μg/kg and 400 ± 96 μg/kg and exceeded the EU regulations 26-and 33-fold. Samples of WF contained 74 ± 28 μg/kg of BaP and 478 ± 164 μg/kg PAH4, which exceeded EU regulations 37-and 40-fold. In comparison, a former study reported mean concentrations of BaP in the range of 8.5–74 μg/kg in traditionally smoked sardines (*Sardinella aurita*) sampled from fishing communities along Ghana's coastal belt (Essumang et al., 2012). However, the skin was removed prior to analysis by Essumang et al. (2012), which resulted in an average 34% reduction of PAH4 in the present study (including removal of the head).

The relative proportion of the individual 15 + 1 EU priority PAH congeners were calculated from mean and UB levels for each tissue of European anchovy (Fig. 3). In general, the different tissues displayed similar PAH-profiles with the PAH4 congeners Chrysene (24–25%), benz[*a*]anthracene (19–22%), benzo[*b*]fluoranthene (9–10%) and BaP (8–10%) representing 62–64% of the 15 + 1 EU priority PAHs. Interestingly, this demonstrated that the percentage of PAH4 was almost equally present in different tissues, indicating that removal of heads and skin is mainly advantageous in terms of total PAH-load in smoked fish.

The calculated MOEs for BaP and PAH4 were markedly lower for all tissues of smoked European anchovy than the threshold of 10 000 determined by EFSA (Fig. 4) and can therefore be assumed to pose a risk to the health of young children. Elevated PAH levels have also been determined in breast milk samples from Ghanaian mothers living near the Agbogbloshie e-waste site, thus adding to the overall toxicant exposure of nursing children (Asamoah et al., 2019). Scientific publications on health implications associated with PAH exposure for young children is limited, but it has been documented that prenatal exposure to high airborne PAH levels is associated with reduced cognitive development (Perera et al., 2006). Furthermore, calculations of excess cancer risk based on lifetime PAH intakes also found that the risks for children were consistently four to seven times higher in magnitude than for adults, underscoring that these health issues should be paid more attention (Xia et al., 2010).

To mitigate the widespread PAH-pollution determined in smoked Ghanaian fish in the current and previous studies (Aheto et al., 2017; Amponsah et al., 2018; Essumang et al., 2012; Hasselberg, Wessels, et al., 2020), a thorough revision of local smoking practices and implementation of relevant codes of practices is imperative. As reviewed by Asamoah et al. (2021), multiple studies have compared the PAHs in smoked fish using different technologies in Ghana. A substantial reduction of BaP and PAH4 in fish smoked in recently developed kilns has been reported (Asamoah et al., 2019), however, traditional smokers are still the most widespread and adoption of new technologies is dependent on accessibility (e.g. cost) and cultural acceptability of the fish products.

3.3. Risk-benefit

Risk-benefit assessments aim to estimate the benefits and risks following exposure and combine them in a stepwise approach, as described by EFSA (EFSA, 2010b). The current study is limited to an initial assessment, where the question of whether the health risks clearly outweigh the health benefits or vice versa is addressed. In our reference scenario it was determined that samples of smoked European anchovy containing HS had the highest concentrations of nutrients, PAHs and metals followed by WF and lastly, WHS-samples. To account for the risks of Cd exposure, daily portion sizes would need to be reduced to approximately 10 g for HS and WF samples in order not to exceed the TWI. However, this reduction would also limit potential nutrient intakes. The MOEs illustrated high levels of BaP and PAH4 (Fig. 4) in all tissues of smoked European anchovy, indicating a concern for consumer health. To achieve an acceptable MOE (>10 000) for BaP and PAH4, daily consumption would need to be restricted to < 1 g for all tissues, which would effectively invalidate the nutritive potential.

Recent risk-benefit publications have considered the potential health effects from substitution of foods in a total diet approach (Pires et al., 2019). This approach is pertinent for the nutrition security of young children in Ghana and other LMICs, since the access and availability of ASFs often are limited. Smoked small fish are available throughout Ghana and can be purchased in small quantities at relatively low costs, whereas ASFs including eggs, dairy and meat are less accessible for low-income households (Akuffo et al., 2020). Additionally, unprocessed ASFs require cooling facilities to keep, which is frequently missing in rural households and therefore represents other food safety hazards including bacterial contamination (Hasselberg, Wessels, et al., 2020; Kombat et al., 2013). Substituting smoked small fish with other ASFs, or plant-based foods in particular, would have a considerable impact on the dietary intakes of multiple nutrients. Given the high prevalence of micronutrient deficiencies among young children in Ghana, caution must be exercised when weighing the risks of consuming certain foods versus the risks of nutrient deficiencies. Indeed, the link between low ASF consumption and micronutrient deficiencies must be emphasized in this scenario and promoting improvements in processing techniques or using alternative processing methods such as drying, rather than substitution, remains a promising approach.

In sum, smoked European anchovy presented both levels of beneficial nutrients important for a healthy diet, as well as detrimental contaminants above recommended values. A refined risk-benefit assessment of smoked fish in Ghana is therefore warranted, however, this will require supplementary data on fish consumption patterns. Furthermore, non-dietary factors including increased toxicant exposure for children living in polluted areas of Ghana should be considered in future risk-benefit assessments.

3.4. Strengths and limitations

The current study provides analytical data on levels of nutrients and contaminants in different tissues of smoked European anchovy from five regions in Ghana. Yet, certain limitations are recognized. By pooling samples from each location, variations between batches may have been masked, and the small sample size (n = 15) entails low statistical power. Furthermore, samples were deskinned and beheaded by hand, and fractions of HS may be present in WHS samples and vice versa. The texture also made homogenization of wet sample material challenging, and unidentified interference during HPLC vitamin A-analysis led to the exclusion of these data from the scenario. Multiple pre-and post-harvest factors affect the distribution of contaminants in Ghana, specifically PAHs and heavy metals, but the current study design did not enable source location. However, the study provides a contemporary indication of the current risks and benefits associated with consuming different tissues of smoked European anchovy in Ghana.

4. Conclusion

In this study, we determined that different tissues of smoked European anchovy contain high levels of nutrients and represent a promising food-based approach to alleviate micronutrient deficiencies in Ghana. However, the current levels of detrimental contaminants above recommended limits will entail potential risk for the consumer, and additional data on dietary intakes-and composition of smoked fish products are needed to perform a refined risk-benefit assessment. Both the current and future generation of risk-benefit data is essential to inform health policies and secure safe utilization of smoked fish in Ghana.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Astrid Elise Hasselberg: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Ole Jakob Nøstbakken:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Supervision. **Inger Aakre:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Supervision. **Lise Madsen:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition. **Amy Atter:** Conceptualization, Writing – review & editing, Project administration, Funding acquisition. **Matilda Steiner-Asiedu:** Conceptualization, Writing – review & editing. **Marian Kjellevold:** Conceptualization, Methodology, Validation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary data

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