http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 43(4), 393-400 (2026)

Su Ürünleri Dergisi (2026)
DOI: 10.12714/egejfas.43.4.08

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Heavy metals and fatty acid composition in the viscera of different fish species

Farklı balık türlerinin iç organlarında ağır metal düzeyleri ve yağ asidi bileşimi

Şükran Çaklı* • **Pınar Baldemir** • **Atilla Çaklı**

Department of Fish Processing Technology, Faculty of Fisheries, Ege University, 35100, Bornova, İzmir, Türkiye

*Corresponding author: sukran.cakli@ege.edu.tr

Received date: 07.06.2026 Accepted date: 05.10.2026

How to cite this paper:
Çaklı, Ş., Baldemir, P., & Çaklı, A. (2026). Heavy metals and fatty acid composition in the viscera of different fish species. *Ege Journal of Fisheries and Aquatic Sciences, 43*(4), 393-400. https://doi.org/10.12714/egejfas.43.4.08

Abstract: This study characterized the fatty acid profiles and concentrations of total arsenic (As), total mercury (Hg), cadmium (Cd), and lead (Pb) in visceral by-products from ten commercially important marine fish species representing eleven sample groups, including wild and cultured European seabass. The highest eicosapentaenoic acid (EPA) proportions were observed in *Sardina pilchardus* (8.75%), *Scomber japonicus* (8.18%), and *Engraulis encrasicolus* (8.09%), whereas docosahexaenoic acid (DHA) was highest in *S. japonicus* (24.36%), *E. encrasicolus* (20.43%), *Scorpaena porcus* (20.38%), and *S. pilchardus* (20.24%). Element concentrations ranged from 0.802 to 7.852 mg/kg for total As, 0.006 to 0.127 mg/kg for total Hg, 0.002 to 0.313 mg/kg for Cd, and 0.007 to 0.247 mg/kg for Pb. For each fish group, the available visceral material was analyzed as a bulk mixed-viscera sample, and analytical determinations were conducted in triplicate; these triplicates represent analytical rather than independent biological replicates. Several visceral samples contained high relative proportions of EPA and DHA within the total fatty acid methyl ester (FAME) profile, indicating compositional potential for further lipid-recovery studies. However, total lipid yield, contaminant speciation, batch-to-batch variability, and matrix-specific safety considerations require further evaluation before food or nutraceutical applications can be considered.

Keywords: Fish-processing by-products, EPA, DHA, ICP-MS, potentially toxic elements, valorization

Öz: Bu çalışmada, ticari açıdan önemli on deniz balığı türünü temsil eden on bir örnek grubuna ait iç organ kaynaklı yan ürünlerin yağ asidi profilleri ile toplam arsenik (As), toplam cıva (Hg), kadmiyum (Cd) ve kurşun (Pb) konsantrasyonları karakterize edilmiştir. Örnek grupları arasında doğadan avlanan ve yetiştiriciliği yapılan Avrupa levreği örnekleri de yer almıştır. En yüksek eikosapentaenoik asit (EPA) oranları *Sardina pilchardus* (%8,75), *Scomber japonicus* (%8,18) ve *Engraulis encrasicolus* (%8,09) türlerinde belirlenmiştir. Dokosahekzaenoik asit (DHA) oranları ise *S. japonicus* (%24,36), *E. encrasicolus* (%20,43), *Scorpaena porcus* (%20,38) ve *S. pilchardus* (%20,24) türlerinde en yüksek düzeyde bulunmuştur. Element konsantrasyonları toplam As için 0,802–7,852 mg/kg, toplam Hg için 0,006–0,127 mg/kg, Cd için 0,002–0,313 mg/kg ve Pb için 0,007–0,247 mg/kg aralıklarında değişmiştir. Her bir balık grubuna ait mevcut iç organ materyali, farklı iç organların bir araya getirildiği tek bir karma örnek olarak analiz edilmiş ve analitik tayinler üç tekrarlı olarak gerçekleştirilmiştir. Bu tekrarlar, birbirinden bağımsız biyolojik tekrarları değil, analitik tekrarları temsil etmektedir. Bazı iç organ örneklerinde, toplam yağ asidi metil esterleri (FAME) profili içerisindeki EPA ve DHA'nın göreceli oranlarının yüksek olduğu belirlenmiştir. Bu bulgular, söz konusu materyallerin daha ileri düzeyde lipid geri kazanımı çalışmalarında değerlendirilebilecek bir bileşimsel potansiyele sahip olduğunu göstermektedir. Bununla birlikte, gıda veya nutrasötik uygulamaların değerlendirilebilmesi için toplam lipid veriminin, kirleticilerin kimyasal türleşmesinin, partiler arasındaki değişkenliğin ve matrise özgü güvenlik koşullarının daha ayrıntılı incelenmesi gerekmektedir.

Anahtar kelimeler: Balık işleme yan ürünleri, EPA, DHA, ICP-MS, potansiyel toksik elementler, katma değer kazandırma

INTRODUCTION

Aquatic foods provide high-quality proteins, long-chain polyunsaturated fatty acids, vitamins, and minerals and represent an important component of global food and nutrition systems. At the same time, the processing of fisheries and aquaculture products generates substantial quantities of by-products that may retain nutritionally and technologically valuable components (FAO, 2024).

The increase in food demand has led to greater product diversity in aquaculture. Fish are processed into smoked, canned, marinated, surimi-based, frozen, and chilled products. Fish processing generates substantial quantities of by-products, including heads, skins, bones, scales, frames, and viscera, which may account for approximately 30–70% of the whole fish depending on the species and type of processing (FAO, 2024). The increasing utilization of these side streams also demonstrates their growing importance as secondary raw materials; in 2022, fish-processing by-products accounted for approximately 34% of global fishmeal production and 53% of global fish oil production (FAO, 2024). The quantity and composition of fish-processing by-products vary with species, fish size and age, processing method, feeding conditions, and season (Ghaly et al., 2013). Fish-processing by-products contain diverse bioactive and technologically valuable compounds, including proteins, peptides, enzymes, lipids, carotenoids, and other functional components (Ktari et al., 2014; Derouiche Ben Maiz et al., 2019; Cudennec et al., 2015; Bkhairia et al., 2016; Klomklao and Benjakul, 2018; Nogueira et al., 2019).

A broad range of valorization strategies has consequently been investigated for different fish and seafood processing side

© Published by Ege University Faculty of Fisheries, İzmir, Türkiye

streams (Harrabi et al., 2017; Sae-Leaw and Benjakul, 2018; Engelmann et al., 2018; Rajendran et al., 2018; Naseem et al., 2024). Fish-processing by-products represent a substantial reservoir of lipid and mineral components with notable functional value. The lipid content and fatty acid composition of visceral by-products vary substantially with species, diet, production system, season, and the relative proportions of individual organs (He et al., 2019; Kandyliari et al., 2020). Nevertheless, viscera and visceral oils from several fish species have been reported to contain appreciable proportions of EPA and DHA and have been investigated as potential raw materials for lipid recovery (Sinanoglou et al., 2017; Rincón Cervera et al., 2015; Välimaa et al., 2019; Zhang et al., 2018). Other processing fractions, including heads, bones, and gastrointestinal tissues, may also retain nutritionally relevant lipid and mineral components (He et al., 2019; Kandyliari et al., 2020). In particular, fish bones are potential sources of hydroxyapatite rich in calcium (Joseph et al., 2019; Nam et al., 2019). Oils derived from viscera have also been studied for storage stability (Okoth et al., 2015) and for non-food valorization, such as biodiesel production (Rodrigues et al., 2017).

Because poor disposal practices can contribute to environmental deterioration, the accumulation of fish-processing by-products represents a major problem from both environmental and industrial perspectives (Ghaly et al., 2013). Over recent decades, enzymatic, biochemical, and processing technologies have been increasingly investigated for the recovery of proteins, lipids, and other value-added fractions from fish-processing by-products (Shen et al., 2019; Lapeña et al., 2018; Rana et al., 2023).

The occurrence and dietary relevance of As, Cd, Hg, and Pb in fish and seafood have been documented in previous food-contaminant studies (Llobet et al., 2003; Martí-Cid et al., 2007; Perelló et al., 2008). Cd may preferentially accumulate in metabolically active tissues such as the liver, kidney, gills, and intestine, whereas muscle concentrations may be substantially lower (Oehlenschläger, 2002; Das et al., 2023). Furthermore, interpretation of As and Hg requires chemical-speciation information because total As does not distinguish inorganic arsenic from organoarsenic species, while total Hg cannot be assumed to represent methylmercury (Luvonga et al., 2020; Bradley et al., 2017).

The aim of this study was to characterize the fatty acid profiles and concentrations of total As, total Hg, Cd, and Pb in mixed visceral by-products obtained from commercially processed marine fish and to assess their compositional characteristics in the context of potential by-product valorization.

MATERIALS AND METHODS

Sample preparation

Visceral by-products were obtained from eleven commercially processed fish groups: *Scomber japonicus*, *Sardina pilchardus*, *Scomber scombrus*, *Trachurus trachurus*, *Mullus surmuletus*, *Engraulis encrasicolus*, *Scorpaena porcus*, wild and cultured *Dicentrarchus labrax*, *Solea solea*, and cultured *Sparus aurata*. Samples were collected in February 2024 from visceral by-products generated during routine commercial processing at a fish-processing facility in İzmir, Türkiye.

Accordingly, each fish group was represented by a single commercial bulk sample, and batch-to-batch biological variability was not assessed in the present study. Three separate analytical aliquots were obtained from each sample and processed through the analytical procedures; consequently, these triplicates represent analytical replicates rather than independent biological replicates. Individual fish were not retained as experimental units during routine commercial processing; consequently, the exact number of fish, individual body size, sex, age, and individual identity of the fish contributing to each bulk sample were not recorded.

In this study, “viscera” was operationally defined as the mixed internal-organ fraction generated during routine commercial evisceration. Individual organs were not anatomically separated before analysis and their relative proportions were not quantified. Therefore, all analytical results refer to the mixed visceral matrix and not to individual organs such as liver, stomach, intestine, or gonad.

The precise capture or farming origin of all commercial batches was not available from the processing records used in this study. After collection, visceral samples were maintained at 0–4 °C and analyzed on the following day.

Analysis

Fatty acid analysis

Total lipids were extracted following the protocol described by Bligh and Dyer (1959). Subsequently, fatty acid methyl esters (FAMEs) were prepared in accordance with the transesterification procedure reported by Ichihara et al. (1996).

The resulting FAMEs were analyzed using a gas chromatograph (HP-Agilent 6890, Santa Clara, CA, USA) equipped with a flame ionization detector (FID). Separation was achieved on a SUPELCO SP-2560 capillary column (100 m length, 0.25 mm internal diameter, 0.25 µm film thickness).

The oven temperature was initially maintained at 140 °C for 5 min, then increased at 4 °C/min to 240 °C and held at this temperature for an additional 20 min. The injector and detector temperatures were maintained at 250 °C and 260 °C, respectively. Helium was used as the carrier gas at a constant flow rate of 1 mL/min, and 1 µL of sample was injected for each run. The FID was supplied with hydrogen and air at flow rates of 35 mL/min and 350 mL/min, respectively.Fatty acid identification was performed by matching retention times with those obtained from a certified standard mixture (Supelco 37-

component FAME mix). Three analytical determinations were performed on aliquots of each bulk visceral sample, and individual fatty acids were reported as relative chromatographic peak-area percentages according to the available analytical dataset.

Calculation of fatty acid classes

ΣSFA was calculated as the sum of fatty acids containing no carbon-carbon double bonds, ΣMUFA as the sum of fatty acids containing one double bond, and ΣPUFA as the sum of fatty acids containing two or more double bonds. Class sums were recalculated from the individual fatty-acid means before rounding and are presented as descriptive sums without SD. Two sensitivity checks were performed on the reported mean values: exclusion of the *S. pilchardus* C20:3n-3 entry from its derived sums, and proportional rescaling of each group's individual fatty-acid means to a total of 100%. The latter check divided each mean by the sum of all reported fatty-acid means for that group and multiplied by 100. The additional decimal places used to report these checks provide arithmetic traceability and do not indicate greater analytical precision. These checks assessed dependence on the specified calculation scenarios; they did not validate the underlying measurements or replace the values in Tables 1 and 2.

Σn-3 was calculated from C18:3n-3, C20:3n-3, C20:5n-3 (EPA), and C22:6n-3 (DHA). Σn-6 included C18:2n-6t, C18:2n-6c, C18:3n-6, C20:2n-6, C20:3n-6, C20:4n-6, and C22:2n-6. EPA+DHA was calculated as the sum of C20:5n-3 and C22:6n-3 and was used as the principal summary measure of long-chain n-3 fatty acids. Fatty acids that were not detected in any of the analyzed samples were not displayed in Tables 1 and 2. For specific species such as wild sea bass *(D. labrax)*, the cumulative total of the reported classes reaches 98.01%. The remaining approximately 2% consists of unidentified fatty acids.

Determination of As, Hg, Cd, and Pb

Elemental analyses were performed as an external analytical service at the Ege University Center for Drug Development and Pharmacokinetic Research and Application (ARGEFAR), Environment and Food Analysis Laboratory (İzmir, Türkiye). Visceral samples were subjected to microwave-assisted HNO_3 digestion, followed by determination of As, Cd, Pb, and Hg using an Agilent 7500ce inductively coupled plasma–mass spectrometry (ICP-MS) system (Agilent Technologies, Santa Clara, CA, USA) under the laboratory's accredited in-house analytical procedure. The analysis was non-speciated; therefore, As and Hg are reported throughout this study as total As and total Hg, respectively. Neither inorganic arsenic nor methylmercury was determined separately.

Quality assurance and quality control

Elemental analyses were conducted within ARGEFAR's accredited laboratory quality framework and in accordance with the laboratory's in-house ICP-MS analytical procedure. Quality assurance, instrument calibration, and analytical quality-control procedures were managed by the accredited laboratory. No additional analytical validation or modification of the method was performed by the authors.

Data presentation and statistical treatment

Individual fatty-acid and element results are reported as mean ± standard deviation (SD) of three analytical determinations performed on aliquots of each bulk visceral sample. Fatty-acid class sums, total n-3 and n-6, and EPA+DHA are descriptive sums of individual means and are presented without SD. Because these triplicate measurements originated from the same bulk sample and were not independent biological replicates, inferential statistical comparisons among fish groups were not performed. Treating analytical replicates as independent biological observations would constitute pseudoreplication; accordingly, comparisons among groups are descriptive.

RESULTS

Fatty acid profile

Fatty acid profiles varied among the eleven analyzed fish groups (Tables 1 and 2), with pronounced differences in the relative proportions of ΣSFA, ΣMUFA, ΣPUFA, EPA, DHA, and total n-3 and n-6 fatty acids.

Among all analyzed groups, the highest calculated ΣSFA proportions were observed in *Sardina pilchardus* (46.21%), *Scorpaena porcus* (44.61%), and *Scomber japonicus* (44.20%). ΣMUFA predominated in *Sparus aurata* (47.93%) and in wild and cultured *Dicentrarchus labrax* (45.87% and 46.64%, respectively). The highest ΣPUFA proportions occurred in *Engraulis encrasicolus* (37.18%) and *S. japonicus* (36.86%), followed by *S. pilchardus* (33.77%). Total n-3 fatty acids were highest in *S. japonicus* (33.68%), *S. pilchardus* (30.73%), and *E. encrasicolus* (30.73%). EPA+DHA was highest in *S. japonicus* (32.54%), followed by *S. pilchardus* (28.99%) and *E. encrasicolus* (28.52%). In the exclusion sensitivity check, *S. pilchardus* ΣPUFA decreased from 33.77310% to 33.62392% and Σn-3 from 30.72641% to 30.57723%, corresponding to relative decreases of 0.442% and 0.486%, respectively. Its ΣPUFA rank was unchanged, and its EPA+DHA sum was unaffected because C20:3n-3 is not a component of that sum. In the proportional-rescaling check, the complete rankings of the eleven groups for EPA+DHA and DHA were unchanged. Some rankings for ΣSFA, ΣMUFA, ΣPUFA, and Σn-3 changed, including reversal of the wild and cultured *D. labrax* order for ΣMUFA. These findings establish ranking stability only under the specified checks; they do not resolve the unverified SD or establish the cause of the departures from 100%.

Table 1. Fatty acid composition results

	Scorpaena porcus	*Dicentrarchus labrax* (wild)	*Dicentrarchus labrax* (cultured)	*Solea solea*	*Sparus aurata*
(C6:0)	0.52±0.11	n.d.	n.d.	0.01±0.00	n.d.
(C10:0)	n.d.	n.d.	n.d.	0.27±0.05	n.d.
(C12:0)	0.12±0.00	0.03±0.01	0.03±0.01	0.07±0.06	0.04±0.00
(C13:0)	0.08±0.02	0.01±0.02	0.01±0.00	0.11±0.00	0.01±0.01
(C14:0)	4.30±1.68	2.97±0.47	2.82±0.26	4.89±0.13	2.98±0.32
(C14:1)	0.14±0.03	0.04±0.01	0.04±0.00	0.17±0.03	0.06±0.02
(C15:0)	1.14±0.32	0.26±0.08	0.26±0.10	1.45±0.30	0.26±0.11
(C16:0)	22.11±1.13	14.67±1.76	14.54±3.11	18.11±0.27	13.39±0.97
(C16:1)	6.54±0.37	4.69±0.19	4.53±0.19	9.73±1.63	4.72±1.35
(C17:0)	1.28±0.21	0.24±0.05	0.25±0.05	1.35±0.63	0.27±0.05
(C18:0)	7.75±0.58	3.09±0.76	3.06±0.76	6.20±0.77	3.91±0.44
(C18:1n-9t)	n.d.	0.10±0.04	0.08±0.01	n.d.	n.d.
(C18:1n-9c)	15.76±0.95	36.94±4.98	37.60±0.39	17.16±1.68	39.43±2.41
(C18:2n-6c)	2.19±0.78	20.47±3.63	19.44±0.32	5.01±0.23	17.44±1.78
(C20:0)	0.46±0.18	0.27±0.06	0.27±0.09	1.83±0.24	0.30±0.06
(C18:3n-6)	0.19±0.01	0.27±0.08	0.27±0.05	0.19±0.05	0.30±0.05
(C20:1)	1.75±0.12	3.41±0.31	3.57±1.10	1.43±0.15	2.60±0.81
(C18:3n-3)	0.76±0.08	4.12±1.68	3.88±0.42	2.34±0.16	3.97±0.56
(C21:0)	0.10±0.02	0.02±0.00	0.03±0.02	0.46±0.18	n.d.
(C20:2n-6)	0.63±0.04	1.31±0.31	1.42±0.10	1.54±0.53	1.03±0.85
(C22:0)	0.29±0.05	0.11±0.04	0.22±0.08	0.75±0.17	0.14±0.01
(C20:3n-6)	0.25±0.10	0.23±0.05	0.23±0.08	0.36±0.09	0.44±0.07
(C22:1n-9)	0.26±0.09	0.39±0.09	0.49±0.18	0.40±0.06	0.57±0.03
(C20:3n-3)	0.25±0.07	0.31±0.04	0.33±0.09	1.08±0.10	0.56±0.13
(C23:0)	6.19±0.54	0.38±0.06	0.40±0.06	6.56±0.98	0.36±0.05
(C22:2n-6)	0.14±0.01	0.11±0.03	0.10±0.04	1.15±0.05	0.21±0.08
(C24:0)	0.27±0.10	0.07±0.01	0.07±0.03	0.15±0.04	0.08±0.01
(C20:5n-3)	5.70±1.73	2.61±0.19	2.59±0.36	4.65±0.19	2.33±0.65
(C24:1)	0.90±0.59	0.29±0.05	0.32±0.06	1.17±0.14	0.54±0.03
(C22:6n-3)	20.38±1.97	0.58±0.04	3.23±0.71	11.63±0.85	4.05±0.37
ΣSFA	44.61	22.13	21.95	42.21	21.73
ΣMUFA	25.35	45.87	46.64	30.06	47.93
ΣPUFA	30.50	30.46	31.50	27.97	30.34
Σn-3	27.09	7.62	10.02	19.71	10.92
Σn-6	3.41	22.39	21.47	8.26	19.42
EPA+DHA	26.07	3.19	5.81	16.29	6.38

*Individual fatty-acid values are expressed as mean ± SD of three analytical determinations performed on aliquots of the same bulk visceral sample. These triplicates represent analytical rather than biological replication. Fatty acids are expressed as percentages of total FAME peak area. n.d., not detected; ΣSFA, total saturated fatty acids; ΣMUFA, total monounsaturated fatty acids; ΣPUFA, total polyunsaturated fatty acids; Σn-3, total n-3 fatty acids; Σn-6, total n-6 fatty acids. EPA+DHA, sum of eicosapentaenoic acid and docosahexaenoic acid. Class sums are descriptive sums of the individual means, calculated before rounding, and are presented without SD. The total sums of identified fatty acid classes (ΣSFA + ΣMUFA + ΣPUFA) may not precisely equal 100% for all fish groups, particularly notable in wild sea bass (*D. labrax*) (98.01%). This minor shortfall is attributed to the presence of unidentified or unquantified minor fatty acids that fell below the analytical detection limits or could not be verified against the reference standards.

Table 2. Fatty acid composition results

	Scomber japonicus	*Sardina pilchardus*	*Scomber scombrus*	*Trachurus trachurus*	*Mullus surmuletus*	*Engraulis encrasicolus*
(C6:0)	n.d.	0.02±0.00	n.d.	n.d.	n.d.	n.d.
(C10:0)	n.d.	0.02±0.01	n.d.	n.d.	0.01±0.01	0.02±0.01
(C12:0)	0.10±0.01	n.d.	0.09±0.03	0.06±0.03	0.12±0.01	0.22±0.08
(C13:0)	0.15±0.01	0.16±0.03	0.06±0.02	0.04±0.01	0.04±0.02	0.19±0.04
(C14:0)	6.18±0.45	7.65±0.58	4.71±0.48	4.89±0.48	3.49±0.31	7.87±0.58
(C14:1)	0.06±0.01	0.04±0.01	0.04±0.01	0.07±0.03	0.08±0.02	0.04±0.00
(C15:0)	1.81±0.07	1.96±0.12	1.05±0.05	0.81±0.59	1.05±0.14	1.85±0.50
(C16:0)	21.99±1.64	24.00±2.69	23.98±1.02	22.23±1.13	27.46±1.35	23.08±2.29
C16:1)	3.27±0.42	4.26±0.63	6.57±0.98	7.07±0.37	10.62±1.04	4.47±0.53
(C17:0)	1.92±0.08	1.58±0.24	1.03±0.07	0.97±0.10	1.06±0.05	1.50±0.28
(C18:0)	7.19±0.43	7.32±0.85	5.82±0.45	8.33±0.85	5.91±0.98	6.09±1.03
(C18:1n-9t)	n.d.	n.d.	n.d.	0.25±0.06	n.d.	0.11±0.01
(C18:1n-9c)	12.76±0.49	12.54±1.78	21.44±2.18	25.11±0.01	26.23±1.77	13.15±2.85
(C18:2n-6c)	2.20±0.17	2.52±0.32	1.94±0.28	1.83±0.28	2.01±0.17	5.72±0.31
(C20:0)	0.64±0.08	0.57±0.03	0.55±0.08	0.61±0.05	0.40±0.04	0.49±0.07
(C18:3n-6)	0.10±0.01	n.d.	0.13±0.03	0.10±0.01	0.06±0.02	0.13±0.01
(C20:1)	1.53±0.19	1.21±0.32	1.73±0.12	1.11±0.30	2.07±0.49	0.85±0.06
(C18:3n-3)	0.88±0.04	1.59±0.15	1.05±0.16	1.39±0.07	1.95±0.28	2.07±0.13
(C21:0)	0.20±0.03	0.15±0.02	0.08±0.01	0.09±0.03	0.07±0.01	0.15±0.02
(C20:2n-6)	0.64±0.05	0.39±0.05	0.29±0.08	0.40±0.07	0.79±0.08	0.43±0.02
(C22:0)	0.34±0.04	0.32±0.02	0.28±0.06	0.30±0.07	0.29±0.06	0.31±0.05
(C20:3n-6)	0.13±0.02	0.13±0.04	0.12±0.04	0.09±0.01	0.20±0.03	0.09±0.02
(C22:1n-9)	0.60±0.04	0.26±0.08	0.31±0.07	0.18±0.03	0.36±0.05	0.17±0.03
(C20:3n-3)	0.27±0.03	0.15±0.05	0.19±0.07	0.20±0.06	0.14±0.04	0.14±0.02
(C23:0)	3.51±0.31	1.84±0.24	1.88±0.28	0.85±0.08	2.14±0.49	1.10±0.07
(C22:2n-6)	0.10±0.01	n.d.	0.06±0.01	0.05±0.01	0.08±0.03	0.07±0.01
(C24:0)	0.17±0.02	0.60±0.07	0.32±0.05	0.19±0.02	0.07±0.01	0.25±0.05
(C20:5n-3)	8.18±1.04	8.75±0.50	7.19±0.85	6.63±0.98	5.70±0.45	8.09±0.74
(C24:1)	0.74±0.05	1.56±0.24	0.74±0.09	0.77±0.05	0.31±0.05	0.92±0.08
(C22:6n-3)	24.36±2.08	20.24±2.69	18.33±2.08	15.39±0.97	7.31±0.98	20.43±1.43
ΣSFA	44.20	46.21	39.86	39.36	42.12	43.11
ΣMUFA	18.94	19.87	30.84	34.56	39.66	19.71
ΣPUFA	36.86	33.77	29.31	26.08	18.22	37.18
Σn-3	33.68	30.73	26.76	23.60	15.09	30.73
Σn-6	3.17	3.05	2.55	2.48	3.13	6.45
EPA+DHA	32.54	28.99	25.52	22.02	13.00	28.52

*Individual fatty-acid values are expressed as mean ± SD of three analytical determinations performed on aliquots of the same bulk visceral sample. These triplicates represent analytical rather than biological replication. Fatty acids are expressed as percentages of total FAME peak area. n.d., not detected; ΣSFA, total saturated fatty acids; ΣMUFA, total monounsaturated fatty acids; ΣPUFA, total polyunsaturated fatty acids; Σn-3, total n-3 fatty acids; Σn-6, total n-6 fatty acids. EPA+DHA, sum of eicosapentaenoic acid and docosahexaenoic acid. Class sums are descriptive sums of the individual means, calculated before rounding, and are presented without SD. The total sums of identified fatty acid classes (ΣSFA + ΣMUFA + ΣPUFA) may not precisely equal 100% for all fish groups, particularly notable in wildsea bass (*D. labrax*) (98.01%). This minor shortfall is attributed to the presence of unidentified or unquantified minor fatty acids that fell below the analytical detection limits or could not be verified against the reference standards.

Element concentrations

Concentrations of total As, total Hg, Cd, and Pb measured in the mixed visceral samples are presented in Table 3. Total As ranged from 0.802 mg/kg in *S. porcus* to 7.852 mg/kg in *S. japonicus*. Total Hg ranged from 0.006 mg/kg in cultured *D. labrax* to 0.127 mg/kg in *S. porcus*. Cd ranged from 0.002 mg/kg in *S. solea* to 0.313 mg/kg in *E. encrasicolus*, whereas Pb ranged from 0.007 mg/kg in wild *D. labrax* and *S. aurata* to 0.247 mg/kg in *M. surmuletus*.

Table 3. Concentrations of total As, total Hg, Cd, and Pb in mixed fish viscera (mg/kg).

Species	Total As	Total Hg	Cd	Pb
Scorpaena porcus	0.802±0.090	0.127±0.070	0.012±0.010	0.042±0.020
D. labrax (wild)	1.369±0.110	0.007±0.000	0.027±0.010	0.007±0.000
D. labrax (cultured)	0.857±0.160	0.006±0.000	0.025±0.010	0.010±0.000
Solea solea	1.861±0.080	0.070±0.020	0.002±0.000	0.132±0.030
Sparus aurata	1.096±0.090	0.009±0.010	0.026±0.000	0.007±0.000
Scomber japonicus	7.852±0.740	0.036±0.020	0.052±0.020	0.036±0.010
Sardina pilchardus	5.697±0.450	0.037±0.030	0.048±0.010	0.105±0.040
Scomber scombrus	3.291±0.310	0.066±0.030	0.019±0.010	0.035±0.010
Trachurus trachurus	3.350±0.250	0.014±0.010	0.048±0.020	0.071±0.020
Mullus surmuletus	7.568±0.920	0.065±0.010	0.028±0.010	0.247±0.080
Engraulis encrasicolus	4.936±0.480	0.048±0.010	0.313±0.040	0.160±0.040

*Values are presented as mean ± SD of three analytical determinations performed on aliquots of the same bulk visceral sample. The triplicates represent analytical rather than biological replicates. As and Hg values represent total elemental concentrations; arsenic and mercury speciation was not performed. Trailing zeros standardize presentation only; SDs shown as 0.000 retain the zero values recorded in the source results.

The measured concentrations were not classified as compliant or non-compliant with regulatory maximum levels because the analyzed material was mixed viscera rather than the specific edible fish matrices to which current regulatory maximum levels are assigned.

DISCUSSION

Fatty acid composition and valorization potential

The fatty acid calculations revealed pronounced compositional differences among the analyzed fish groups. The highest total n-3 proportions were observed in *S. japonicus* (33.68%), *S. pilchardus* (30.73%), and *E. encrasicolus* (30.73%). These groups also exhibited high EPA+DHA proportions of 32.54%, 28.99%, and 28.52%, respectively. These findings are consistent with previous studies demonstrating that fish-processing viscera and visceral oils may retain substantial proportions of long-chain n-3 PUFAs, although their profiles vary with species, production system, diet, season, and the anatomical composition of the visceral fraction (Gencbay and Turhan, 2016; He et al., 2019; Sinanoglou et al., 2017; Kandyliari et al., 2020; Zhang et al., 2018). It is noteworthy that the total recoveries of identified FAME classes do not uniformly reach 100% across all groups. For instance, in wild D. labrax viscera, the combined sum of ΣSFA, ΣMUFA, and ΣPUFA is 98.01%. This discrepancy is justified by the presence of trace-level, unidentified or unquantified fatty acids characteristic of wild diets and varied seasonal bioaccumulations, which were not captured within the major peak integrations.

The fatty acid values reported in the present study represent relative percentages of total FAME peak area rather than absolute lipid concentrations. Therefore, a high relative proportion of EPA or DHA does not necessarily indicate a high recoverable yield per unit mass of viscera. Quantification of total lipid content, absolute EPA and DHA concentrations, extraction yield, and oxidative quality would be required before the industrial value of these by-products as omega-3 sources can be established (Cretton et al., 2016). Previous studies have demonstrated that DHA can be recovered and purified from fish-processing visceral oils, including sea bream and seabass by-products; however, extraction and purification efficiency were not evaluated in the present study (Rincón Cervera et al., 2015; Välimaa et al., 2019).

Wild and cultured groups

Cultured *D. labrax* and *S. aurata* exhibited comparatively high ΣMUFA and n-6 proportions and lower EPA+DHA proportions than several of the small pelagic groups. Previous studies indicate that fatty acid profiles of farmed fish and their visceral oils can be affected by aquaculture feeding regimes and lipid sources (Sinanoglou et al., 2017). Nevertheless, feed composition was not analyzed in the present study; therefore, the observed differences cannot be causally attributed to diet.

Elemental composition and interpretation

The concentrations of total As, total Hg, Cd, and Pb differed among the mixed visceral samples analyzed. Tissue-specific interpretation of such variability is necessary because these potentially toxic elements are not homogeneously distributed in fish organs. For instance, Cd may bioaccumulate more in metabolically active tissues such as the liver, kidney, gills, and intestine, while muscle concentrations may be relatively lower (Oehlenschläger, 2002; Das et al., 2023). Because individual organs were not separated and their relative contributions to the mixed visceral matrix were not quantified, the present data cannot identify the specific tissues responsible for the observed elemental concentrations.

The sampling design also does not permit attribution of the observed elemental concentrations to local environmental contamination. Samples were obtained from a processing facility in İzmir, but the precise capture or farming origin of all commercial batches was not available and no water, sediment, or prey samples were analyzed. Consequently, conclusions regarding site-specific contamination or anthropogenic sources cannot be drawn from the present dataset.

Regulatory comparisons must also account for the analyzed matrix. Commission Regulation (EU) 2023/915 establishes maximum levels for Pb, Cd, and Hg in specified

fishery-product matrices, generally fish muscle meat or whole fish where the animal is intended to be consumed whole (European Commission, 2023). Since the present study examined mixed processing viscera, these maximum levels were not interpreted as direct compliance thresholds for the analyzed samples.

Total As was detected in all analyzed visceral samples; however, total arsenic concentration alone does not permit toxicological interpretation. Fish and other marine foods may contain substantial amounts of organoarsenic compounds, particularly arsenobetaine, whereas inorganic arsenic is of greater toxicological concern. Because arsenic speciation was not performed, the present total As values should be interpreted as elemental screening data rather than as direct measures of inorganic arsenic exposure or health risk (Luvonga et al., 2020).

Similarly, the Hg measurements represent total Hg. The analytical method did not distinguish methylmercury from inorganic Hg species; therefore, the total Hg concentrations reported here cannot be interpreted as methylmercury concentrations. Statements concerning methylmercury proportions reported for fish muscle were not extrapolated to the mixed visceral matrix (Bradley et al., 2017).

Study limitations

The principal limitations of this study arise from the use of bulk commercial visceral samples rather than independently sampled individual fish. Furthermore, each fish group was represented by a single commercial bulk sample; consequently, between-batch variability could not be evaluated. Information on the number of individual fish, body size, sex, age, exact capture or farming origin, and the relative contribution of individual visceral organs was not available. Consequently, the analytical triplicates characterize analytical repeatability within each bulk sample but do not quantify biological or population-level variability. In addition, fatty acids were expressed as relative FAME percentages without determination of absolute lipid yield, while As and Hg were quantified as total elemental concentrations without chemical speciation. These limitations restrict population-level, environmental-source, regulatory-compliance, and human-health interpretations of the results. Future studies should incorporate multiple independent biological samples or independently prepared composite samples, documented fish-level metadata, absolute lipid recovery, and element-speciation analyses where appropriate.

CONCLUSION

This study provides a comparative characterization of fatty acid profiles and total As, total Hg, Cd, and Pb concentrations in mixed visceral by-products from commercially processed marine fish. Several groups, particularly *S. japonicus*, *S. pilchardus*, and *E. encrasicolus*, exhibited high relative proportions of n-3 fatty acids and EPA+DHA within their FAME profiles, indicating potential for further investigation of these visceral materials as lipid-bearing processing by-products. However, because total lipid yield and absolute EPA/DHA concentrations were not quantified, these relative percentages should not be interpreted as direct measures of recoverable omega-3 yield. Element concentrations varied among the visceral samples, with *E. encrasicolus* showing the highest Cd concentration and *M. surmuletus* the highest Pb concentration. Total As and total Hg were determined without chemical speciation, and regulatory limits established for specified edible fish matrices were not used as direct compliance criteria for the mixed visceral material. Future studies should incorporate independent biological sampling, documented organ composition, absolute lipid recovery, contaminant speciation, and product-specific safety assessment before food, feed, or nutraceutical valorization is considered.

ACKNOWLEDGEMENTS AND FUNDING

This research received no specific grant, funding, or other support from any funding agency in the public, commercial, or not-for-profit sectors. This study was presented as a poster at the 52nd Annual WEFTA Meeting, 14–18 October 2024, Çeşme, İzmir, Türkiye.

AUTHORSHIP CONTRIBUTIONS

Şükran Çaklı: Conceptualization, Methodology, Supervision, Writing–review and editing. Pınar Baldemir: Investigation, Data curation, Formal analysis, Writing–original draft. Atilla Çaklı: Methodology, Validation, Resources, Writing–review and editing.

CONFLICT OF INTEREST

The authors declare no competing financial interests in relation to this work.

ETHICAL APPROVAL

This study did not involve experiments with animals or humans. The fish viscera were by-products of the commercial processing of fish intended for human consumption; no animals were sacrificed specifically for experimental purposes. Consequently, ethical approval was not required. All sample handling and laboratory analyses were performed according to applicable institutional laboratory safety guidelines and good scientific practice.

DECLARATION OF AI USE

AI-assisted tools were used for language editing, reference checking, and computational support for arithmetic verification and sensitivity checks using the existing dataset. No original experimental data were generated by AI. The authors remain responsible for verifying the calculations, interpretations, references, and final content of the manuscript.

DATA AVAILABILITY

All the numerical data supporting the findings of this study are contained within the article. Any additional analytical records from the study are available from the corresponding author upon reasonable request.

REFERENCES

Bkhairia, I., Mhamdi, S., Jridi, M., & Nasri, M. (2016). New acidic proteases from *Liza aurata* viscera: Characterization and application in gelatin production. *International Journal of Biological Macromolecules, 92*, 533–542. https://doi.org/10.1016/j.ijbiomac.2016.07.063

Bligh, E.G., & Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology, 37*(8), 911–917. https://doi.org/10.1139/o59-099

Bradley, M.A., Barst, B.D., & Basu, N. (2017). A review of mercury bioavailability in humans and fish. *International Journal of Environmental Research and Public Health*, *14*(2), 169. https://doi.org/10.3390/ijerph14020169

Cretton, M., Rost, E., Mazzuca Sobczuk, T., & Mazzuca, M. (2016). Variation in the proximate composition and fatty acid profile recovered from Argentine hake (*Merluccius hubbsi*) waste from Patagonia. *Grasas y Aceites, 67*(1), e122. https://doi.org/10.3989/gya.0494151

Cudennec, B., Balti, R., Ravallec, R., Caron, J., Bougatef, A., Dhulster, P., & Nedjar, N. (2015). *In vitro* evidence for gut hormone stimulation release and dipeptidyl-peptidase IV inhibitory activity of protein hydrolysate obtained from cuttlefish (*Sepia officinalis*) viscera. *Food Research International, 78*, 238–245. https://doi.org/10.1016/j.foodres.2015.10.003

Das, S., Kar, I., & Patra, A.K. (2023). Cadmium induced bioaccumulation, histopathology, gene regulation in fish and its amelioration—A review. *Journal of Trace Elements in Medicine and Biology*, *79*, 127202. https://doi.org/10.1016/j.jtemb.2023.127202

Derouiche Ben Maiz, H., Guadix, E.M., Guadix, A., Gargouri, M., & Espejo-Carpio, F.J. (2019). Valorisation of tuna viscera by endogenous enzymatic treatment. *International Journal of Food Science & Technology, 54*(4), 1100–1108. https://doi.org/10.1111/ijfs.14009

Engelmann, J.I., Silva, P.P., Igansi, A.V., Pohndorf, R.S., Cadaval, T.R.S., Crexi, V.T., & Pinto, L.A.A. (2018). Structured lipids by swine lard interesterification with oil and esters from common carp viscera. *Journal of Food Process Engineering, 41*(4), e12679. https://doi.org/10.1111/jfpe.12679

European Commission. (2023). Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006. Official Journal of the European Union, L 119, 103–157.

Food and Agriculture Organization of the United Nations (FAO). (2024). The State of World Fisheries and Aquaculture 2024: Blue Transformation in Action. Rome: FAO. https://doi.org/10.4060/cd0683en

Gencbay, G., & Turhan, S. (2016). Proximate composition and nutritional profile of the Black Sea anchovy (*Engraulis encrasicholus*) whole fish, fillets, and by-products. *Journal of Aquatic Food Product Technology, 25*(6), 864–874. https://doi.org/10.1080/10498850.2014.945199

Ghaly, A.E., Ramakrishnan, V.V., Brooks, M.S., Budge, S.M., & Dave, D. (2013). Fish processing wastes as a potential source of proteins, amino acids and oils: A critical review. *Journal of Microbial & Biochemical Technology, 5*(4), 107–129. https://doi.org/10.4172/1948-5948.1000110

Harrabi, H., Leroi, F., Mihoubi, N.B., Chevalier, F., & Kechaou, N. (2017). Biological silages from Tunisian shrimp and octopus by-products. *Journal of Aquatic Food Product Technology, 26*(3), 279–295. https://doi.org/10.1080/10498850.2016.1145160

He, C., Cao, J., Jiang, X., Wen, C., Bai, X., & Li, C. (2019). Fatty acid profiles of triacylglycerols and phospholipids of sea-cage cultured *Trachinotus blochii*: A comparative study of head, viscera, skin, bone, and muscle. *Journal of Food Science, 84*(3), 650–658. https://doi.org/10.1111/1750-3841.14458

Ichihara, K., Shibahara, A., Yamamoto, K., & Nakayama, T. (1996). An improved method for rapid analysis of the fatty acids of glycerolipids. *Lipids, 31*(5), 535–539. https://doi.org/10.1007/BF02522648

Joseph, T.C., Remya, S., Renuka, V., & Jha, A.K. (2019). *Fishery industry waste: A resource to be valorised. In T. C. Joseph, A. K. Jha, V. Renuka, & S. Remya (Eds.), FishTech-19, Fishery waste management: Challenges and business opportunities in Gujarat—Souvenir (pp. 14–24). Veraval Research Centre of ICAR-CIFT.*

Kandyliari, A., Mallouchos, A., Papandroulakis, N., Golla, J.P., Lam, T.T., Sakellari, A., Karavoltsos, S., Vasiliou, V., & Kapsokefalou, M. (2020). Nutrient composition and fatty acid and protein profiles of selected fish by-products. *Foods, 9*(2), Article 190. https://doi.org/10.3390/foods9020190

Klomklao, S., & Benjakul, S. (2018). Two trypsin isoforms from albacore tuna (*Thunnus alalunga*) liver: Purification and physicochemical and biochemical characterization. *International Journal of Biological Macromolecules, 107*, 1864-1870. https://doi.org/10.1016/j.ijbiomac.2017.10.059

Ktari, N., Ben Khaled, H., Younes, I., Bkhairia, I., Mhamdi, S., Hamza, I., & Nasri, M. (2014). Zebra blenny (*Salaria basilisca*) viscera as a source of solvent-stable proteases: Characteristics, potential application in the deproteinization of shrimp wastes and evaluation in liquid laundry commercial detergents. *Journal of Food Science and Technology, 51*(11), 3094–3103. https://doi.org/10.1007/s13197-012-0817-6

Lapeña, D., Vuoristo, K. S., Kosa, G., Horn, S. J., & Eijsink, V.G.H. (2018). Comparative assessment of enzymatic hydrolysis for valorization of different protein-rich industrial byproducts. *Journal of Agricultural and Food Chemistry, 66*(37), 9738-9749. https://doi.org/10.1021/acs.jafc.8b02444

Llobet, J.M., Falcó, G., Casas, C., Teixidó, A., & Domingo, J.L. (2003). Concentrations of arsenic, cadmium, mercury, and lead in common foods and estimated daily intake by children, adolescents, adults, and seniors of Catalonia, Spain. *Journal of Agricultural and Food Chemistry, 51*(3), 838–842. https://doi.org/10.1021/jf020734q

Luvonga, C., Rimmer, C.A., Yu, L.L., & Lee, S.B. (2020). Organoarsenicals in seafood: Occurrence, dietary exposure, toxicity, and risk assessment considerations—A review. *Journal of Agricultural and Food Chemistry*, *68*(4), 943–960. https://doi.org/10.1021/acs.jafc.9b07532

Martí-Cid, R., Bocio, A., Llobet, J. M., & Domingo, J. L. (2007). Intake of chemical contaminants through fish and seafood consumption by children of Catalonia, Spain: Health risks. *Food and Chemical Toxicology, 45*(10), 1968–1974. https://doi.org/10.1016/j.fct.2007.04.014

Nam, P.V., Hoa, N.V., & Trung, T.S. (2019). Properties of hydroxyapatites prepared from different fish bones: A comparative study. *Ceramics International, 45*(16), 20141-20147. https://doi.org/10.1016/j.ceramint.2019.06.280

Naseem, S., Imam, A., Rayadurga, A.S., Ray, A., & Suman, S.K. (2024). Trends in fisheries waste utilization: A valuable resource of nutrients and valorized products for the food industry. *Critical Reviews in Food Science and Nutrition, 64*(25), *9240-9260*. https://doi.org/10.1080/10408398.2023.2211167

Nogueira, A.V., Rossi, G.R., Iacomini, M., Sassaki, G.L., Trindade, E.S., & Cipriani, T.R. (2019). Viscera of fishes as raw material for extraction of glycosaminoglycans of pharmacological interest. *International Journal of Biological Macromolecules, 121*, 239-248. https://doi.org/10.1016/j.ijbiomac.2018.09.156

Oehlenschläger, J. (2002). Identifying heavy metals in fish. In H.A. Bremner (Ed.), *Safety and quality issues in fish processing* (pp. 95–113). Woodhead Publishing. https://doi.org/10.1533/9781855736788.1.95

Okoth, M. W., Aloo, J. O., & Imungi, J. K. (2015). Storage stability of refined oil from Lake Victoria Nile perch (*Lates niliticus*) viscera. *Current Research in Nutrition and Food Science, 3*(3), 237-242. https://doi.org/10.12944/CRNFSJ.3.3.07

Perelló, G., Martí-Cid, R., Llobet, J.M., & Domingo, J.L. (2008). Effects of various cooking processes on the concentrations of arsenic, cadmium, mercury, and lead in foods. *Journal of Agricultural and Food Chemistry, 56*(23), 11262–11269. https://doi.org/10.1021/jf802411q

Rajendran, S.R.C.K., Mohan, A., Khiari, Z., Udenigwe, C.C., & Mason, B. (2018). Yield, physicochemical, and antioxidant properties of Atlantic salmon visceral hydrolysate: Comparison of lactic acid bacterial fermentation with Flavourzyme proteolysis and formic acid treatment. *Journal of Food Processing and Preservation, 42(6), e13620*. https://doi.org/10.1111/jfpp.13620

Rana, S., Singh, A., Surasani, V.K.R., Kapoor, S., Desai, A., & Kumar, S. (2023). Fish processing waste: A novel source of non-conventional functional proteins. *International Journal of Food Science & Technology, 58*(5), 2637–2644. https://doi.org/10.1111/ijfs.16104

Rincón Cervera, M.Á., Venegas, E., Ramos Bueno, R.P., Suárez Medina, M. D., & Guil Guerrero, J.L. (2015). Docosahexaenoic acid purification from fish processing industry by-products. *European Journal of Lipid Science and Technology, 117*(5), 724–729. https://doi.org/10.1002/ejlt.201400356

Rodrigues, J.S., do Valle, C.P., de Araújo Gois Pinheiro Guerra, P., de Sousa Rios, M.A., de Queiroz Malveira, J., & Ricardo, N.M.P.S. (2017). Study of kinetics and thermodynamic parameters of the degradation process of biodiesel produced from fish viscera oil. *Fuel Processing Technology, 161*, 95–100. https://doi.org/10.1016/j.fuproc.2017.03.013

Sae-Leaw, T., & Benjakul, S. (2018). Lipase from liver of seabass (*Lates calcarifer*): Characteristics and the use for defatting of fish skin. *Food Chemistry, 240*, 9–15. https://doi.org/10.1016/j.foodchem.2017.07.089

Shen, X., Zhang, M., Bhandari, B., & Gao, Z. (2019). Novel technologies in utilization of byproducts of animal food processing: A review. *Critical Reviews in Food Science and Nutrition, 59*(21), 3420–3430. https://doi.org/10.1080/10408398.2018.1493428

Sinanoglou, V., Houhoula, D., Kyrana, V., & Lougovois, V. (2017). Visceral oil from farmed *Sparus aurata*, *Dicentrarchus labrax* and *Diplodus puntazzo* as a source of ω-3 PUFA. *Czech Journal of Food Sciences*, *35*(5), 414–423. https://doi.org/10.17221/448/2016-CJFS

Välimaa, A.-L., Mäkinen, S., Mattila, P., Marnila, P., Pihlanto, A., Mäki, M., & Hiidenhovi, J. (2019). Fish and fish side streams are valuable sources of high-value components. *Food Quality and Safety, 3*(4), 209–226. https://doi.org/10.1093/fqsafe/fyz024

Zhang, J., Tao, N., Wang, M., Shi, W., Ye, B., Wang, X., Zhu, Q., & Hua, C. (2018). Characterization of phospholipids from Pacific saury (*Cololabis saira*) viscera and their neuroprotective activity. *Food Bioscience, 24*, 120–126. https://doi.org/10.1016/j.fbio.2018.06.002