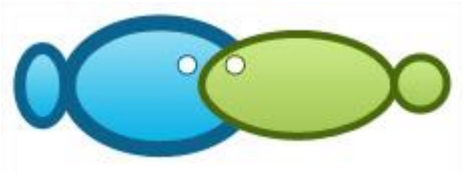

Analysing fatty acid profiles and quality indices of different puffer fish species of east Malaysia waters

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Abstract. Despite containing tetrodotoxin, which renders puffer fish inedible and often classifies them as trash fish, these species possess valuable fatty acids, specifically omega-3 fatty acids, which are associated with substantial health benefits. In the present study, the liver and muscles of five distinct puffer fish species namely *Xenopterus naritus*, *Diodon hystrix*, *Diodon holocanthus*, *Lactoria cornuta*, and *Rhynchostracion nasus* were examined for their fatty acid content, physicochemical properties, composition, and quality indices. Fatty acids extraction from puffer fish tissues was performed using the cooking-pressing technique, and physicochemical properties of the extracted lipids were determined using the standard AOCS official method. In the liver, fatty acids were between 21.10 and 27.50%, but they were much lower in the muscles, between 0.50 and 1.30%. A comprehensive analysis using gas chromatography identified a total of 22 distinct fatty acids. Saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) levels in the liver were determined to be 31.52-35.43%, 27.5-37.87%, and 14.63-21.95%, respectively. Muscle, on the other hand, had SFA, MUFA, and PUFA contents of 16.67-19.16%, 24.23-21.00%, and 5.4-12.11%, respectively. The lipid from *R. nasus*, both from its liver and muscle, exhibited the most beneficial fatty acid profile due to its highest PUFA/SFA and EPA+DHA levels, along with the lowest omega-6/omega-3 ratio. All studied species had values of atherogenic (IA) and thrombogenic (IT) indices lower than 1. *X. naritus* showed the lowest free fatty acid (FFA) and acid value (AV) levels in both its liver and muscle. *D. hystrix* showed the highest PV and IV values, while *R. nasus* had the highest SV value. This study highlights the significance of puffer fish as a potential source of valuable fatty acids, particularly omega-3 fatty acids, offering essential health benefits and nutritional value.
Key Words: fatty acid profiles, quality indices, puffer fish lipid, omega-3, polyunsaturated fatty acid.

Introduction. Puffer fish, known for containing the potent toxin tetrodotoxin (TTX), have been a long-standing public health concern. This toxin is most concentrated in their skin and internal organs, including the ovaries and livers (Madejska et al 2019). Notably, up until the year 2020, a significant percentage (93.7%) of the 1,703 TTX-intoxication cases were attributed to pufferfish and other fish within the Tetraodontiformes order, while the rest were caused by porcupinefish, boxfish, gobies, or unidentified fish (Guardone et al 2020). In the states of Sarawak and Sabah in Malaysia, various species of puffer fish, including the yellow puffer fish, black-spotted porcupine fish, long-spined porcupine fish, long-horned cowfish, and shortnose boxfish, can be found in wet markets and are highly regarded as edible exotic foods. However, their nutritional value is often underestimated, leading some fishermen to regard them as trash fish (Mohd Nor Azman et al 2015). Despite this perception, like other marine organisms, puffer fish offer significant nutritional value through their fish lipids.

Fish lipids are rich in fatty acids and play vital roles in human health. These fatty acids can be categorized as saturated (SFAs), monounsaturated (MUFAs), and polyunsaturated (PUFAs). Their effects on health vary, underscoring the importance of

consuming them in appropriate quantities (Rustan & Drevon 2005). Maintaining a healthy body involves reducing SFA intake, as it adversely affects total and LDL-cholesterol levels, diastolic blood pressure, and cardiovascular health, while increasing the consumption of PUFAs (Te Morenga & Montez 2017). Since the human body cannot synthesize PUFAs, including omega-3 fatty acids such as DHA and EPA, they must be obtained through the diet. Extensive epidemiological studies and clinical trials have demonstrated the preventive and therapeutic potential of omega-3 PUFAs against various diseases (Ander et al 2003). These fatty acids possess anti-inflammatory properties, beneficial in combating conditions like cardiovascular disease, cancer, and Alzheimer's disease (Wall et al 2010; Troesch et al 2020). Omega-3 PUFAs also help lower the risk of cardiovascular disease by reducing triglycerides, blood pressure, and the formation of blood clots (Breslow 2006). Furthermore, their anti-cancer properties are particularly notable in preventing breast, prostate, and colon cancer (Jing et al 2013; Weitz et al 2010). These fatty acids play a vital role in brain health, cognitive function, mood regulation, and can aid in preventing age-related macular degeneration, a leading cause of blindness in older adults (Merle et al 2014). Specifically, DHA and EPA are essential components for brain and neurological tissue development, with studies recommending their inclusion in the diet during the last trimester of pregnancy and infant feeding to support organ growth (Pike & Jackson 2010).

Apart from the well-established health benefits of omega-3 PUFAs, researchers are exploring alternative sources of these valuable fatty acids. By harnessing the potential health benefits of PUFAs found in puffer fish lipids, researchers believe they could offer a valuable source for human consumption, further enhancing the positive impact of omega-3 PUFAs on human health (Hazra et al 1998; Aydin et al 2013; Oyaizu et al 2000; Kaleshkumar et al 2022). While several prior studies have investigated the fatty acid composition of puffer fish, there remains a lack of comprehensive data on the physicochemical properties and quality indices of puffer fish lipids, which can provide insights into their suitability for human consumption. Therefore, this study aimed to determine the fatty acid composition, physicochemical properties, and various quality indices of *Xenopterus naritus*, *Diodon hystrix*, *Diodon holocanthus*, *Lactoria cornuta*, and *Rhynchostracion nasus*, puffer fish lipids extracted from their livers and muscles.

Material and Method

Sample collection and preparation. During field sampling in September 2022, liver and muscle samples were obtained from five puffer fish species, which included *X. naritus*, *D. hystrix*, *D. holocanthus*, *L. cornuta*, and *R. nasus*. These puffer fish samples were purchased from wet markets in Samarahan, Sarawak, and Kota Kinabalu, Sabah (Figure 1).

Figure 1. Puffer fish commonly found in wet markets, Samarahan, Sarawak and Kota Kinabalu Sabah (a) *Xenopterus naritus* are sold together with other fishes; (b) Price per kg for *Xenopterus naritus* is RM 12 (2.5 USD kg^{-1}); (c) *Diodon holocanthus* and (d) *Diodon hystrix* can be found in wet markets considered as exotic seafoods.

The collected samples were immediately packed in plastic bags, placed on ice, and transported to the laboratory in cooler boxes to preserve their freshness. Subsequently, the samples were stored at -20°C until analysis. All chemicals and reagents used in the study were of analytical grade.

Lipid extraction. The extraction of fish lipids was performed using the cooking-pressing method with some modifications based on Inguglia et al (2020). Approximately 20 g of each sample (liver and muscle) was homogenized separately and boiled with 1:1 volume of distilled water at 85-90°C for 1 hour. After boiling, the mixture was pressed using an automatic screw expeller machine (Kubota 2000, Japan) to separate any remaining lipid. The resulting lipid was then centrifuged at 5,000 rpm for 10 minutes, and the top layer containing the lipid was collected for further processing.

Lipid refining. The lipid refining process was carried out in three stages: degumming, neutralization, and bleaching, following the method described by Nazir et al (2017) with minor modifications. (a) Degumming: The lipid was stirred and heated at 70°C. Hot water was added to the lipid at a volume of 10% of the lipid's volume, and the mixture was then centrifuged at 3000 rpm for 5 minutes to form three layers: lipid, gum, and water. The lipid layer was collected, and the process was repeated until the water reached a neutral pH. (b) Neutralization: The degummed lipid was stirred and heated to 80°C. Then, 20% w/w of potassium hydroxide (KOH) solution was added to the lipid and stirred for 2 minutes. The mixture was transferred to a centrifuge tube and centrifuged at 3000 rpm for 5 minutes to form three layers: lipid, soap stock, and water. The lipid layer was collected, and the process was repeated until the water reached a neutral pH. (c) Bleaching: The neutralized lipid was heated to 80-100°C and mixed with 1% active charcoal, stirred for 10 minutes, and then filtered using a Buchner funnel and filter paper.

Determination of yield. The lipid yield was calculated as the percentage of extracted fish lipid from the livers and muscles of puffer fish, using the formula (Nazri et al 2017):

$$\text{Yield (\%)} = [\text{Extracted fish lipid weight (g)} / \text{Weight of sample (g)}] \times 100$$

Gas chromatography analysis. The fish lipids were methylated to their fatty acid methyl esters (FAMEs) following the procedure described by Ichihara et al (1996). Approximately 20 mg of lipid sample was dissolved in 2 mL n-heptane in a centrifuge tube and mixed with 4 mL of 2 M methanolic KOH. The mixture was vortexed for 2 minutes at room temperature at 4000 rpm for 10 minutes, and the solution was allowed to separate into a clear-colored FAME solution and a cloudy aqueous layer. The top layer containing the FAMEs was collected and analyzed using a Shimadzu Gas Chromatography-Mass Spectrometer (GC-MS) model QP2010plus equipped with a 30 m x 0.25 mm x 250 µm DB5 column and an auto-sampler. The methylated lipid samples were directly injected into the column with an initial oven temperature of 50°C for 10 minutes, followed by a ramp up to 350°C at a rate of 4.5°C min^{-1}, and then held at the final temperature for 10 minutes. Helium was used as the carrier gas at a flow rate of 1.0 mL min^{-1}. Peaks were identified using the retention times from a standard 37 component FAME mix (CRM47885).

Physicochemical properties analyses. The determination of Free Fatty Acid (FFA) and Acid Value (AV) was conducted using the approved AOCS Official Method Ca 5a-40. FFA was quantified as a percentage of oleic acid through titration with a 0.25N NaOH solution. The (AV) was derived by multiplying the FFA percentage by 1.99, and the Saponification Value (SV) was determined using AOCS Method Cd 3-25 (1993) through titration with a 0.5N HCl solution. The Peroxide Value (PV) was measured in milliequivalents per kilogram using AOCS Official Method Cd 8-53, and the Iodine Value (IV) was determined in grams per 100 grams of sample following AOCS Method Cd 1-25 with Wijs solution.

Determination of quality indices. The nutritional value of the lipid fraction was evaluated based on the fatty acid composition data, and five quality indices were calculated:

i. Polyunsaturated fatty acid/saturated fatty acid ratio (PUFA/SFA): (∑PUFA)/(∑SFA)
ii. Sum of EPA and DHA (EPA+DHA): C22:6n-3+C20:5n-3
iii. Omega-6 to Omega-3 ratio: (∑Omega-6) / (∑Omega-3)
iv. Index of atherogenicity (IA): IA = [C12:0+(4 × C14:0)+C16:0] /ΣUFA
v. Index of thrombogenicity (IT): IT = [C14:0+C16:0+C18:0] / [(0.5 × ΣMUFA)+(0.5 × Σn-6)+(3 × Σn-3)+(n-3/n-6)]

Statistical analysis. Each analysis was performed in triplicates, and the reported values represent the mean ± standard deviation of three separate determinations. The one-way analysis of variance (ANOVA) followed by LSD test in SPSS software version 27 was used for statistical analysis, and the significance was defined as $p<0.05$.

Results. The percentage of lipid yield extracted from livers and muscles of different puffer fish species studied are presented in Table 1. The ranges of lipid yield extracted from livers and muscles were 21.10-27.50% and 0.50-1.30% respectively. *L. cornuta* has the highest yield in both the liver and muscle among the species examined. *D. hystrix* and *X. naritus* also exhibit relatively high yields, particularly in the liver. *R. nasus* generally showed the lowest yield among the species studied. Across all species, the liver tends to have a higher lipid yield compared to the muscle. The difference in lipid yield between the liver and muscle varies among species, with *L. cornuta* exhibiting the largest disparity. The significant difference in the yield of puffer fish oil extracted from the liver and muscle is likely due to the higher concentration of lipid in the liver, which is an organ known to accumulate lipids. On the other hand, the muscle is a leaner tissue with fewer lipids, resulting in a lower yield of oil. This study showed higher oil yield extracted from liver than from muscle, in line with previous studies on various species, such as *X. naritus* (0.01-0.77%), reported by Mohd Nor Azman et al (2015), *L. sceleratus* (0.07-0.34%), reported by Aydin et al (2013), *Lagocephalus* (0.93%), reported by Ghosh et al (2005), and 26 species of wild and cultured pufferfish (1.20-2.50%), reported by Oyaizu et al (2000).

Table 1

Lipid yield (%) extracted from liver and muscle of different puffer fish species

Species	*Liver*	*Muscle*
Xenopterus naritus	23.60 ± 0.60^{a}	1.10 ± 0.23^{a}
Diodon hystrix	26.30 ± 0.56^{b}	0.70 ± 0.06^{b}
Diodon holocanthus	23.50 ± 0.41^{a}	0.80 ± 0.17^{b}
Lactoria cornuta	27.50 ± 0.37^{b}	1.30 ± 0.46^{a}
Rhynchostracion nasus	21.10 ± 0.54^{c}	0.50 ± 0.10^{c}

Different superscripts indicate a significant difference at $p<0.05$.

Puffer fish is categorized as 'lean fish' as it accumulates more oil content in liver compared to muscle tissues (Kosker et al 2018). Moreover, the white color of their flesh indicates a low-fat fish containing more water, while flesh with yellow, grey, pink or other colors reflects that fish stores its fat in muscle tissue (Osman et al 2001). Rahnan et al (1995) divided fish, according to their lipid content, into lean (<5%), medium (5-10%) and fatty (>10%), while Abd Aziz et al (2013) and Nurnadia et al (2011), proposed lean (<2%), low fat (2-4%), medium fat (4-8%), and high fat (>8%) fish categories. Based on the species of puffer fish in this study, all livers are fatty but their muscles are lean. Heat applied in cooking-pressing method coagulates the protein of fish tissue, breaking the cell structure to ensure a good permeability for the oil to be extracted (Nazir et al 2017). Moreover, mechanical pressing also assists to separate the oil and water phase from the solid protein (press cake), hence allows the remaining oil to be separated

completely (Bako et al 2017). Apart from that, several factors including stage of fish, habitat, fish species, nutrition, and extraction process might impact the yield differential (Ivanovs & Blumberga 2017). Hazra et al (1998) reported that the seasonal variation could also affect the lipid content in puffer fish liver as they found out the highest was during monsoon season. Besides, tropical marine and freshwater fishes have generally lower oil content as compared to temperate marine fishes (Silva & Chamul 2000). For cultured and wild puffer, Koizumi & Hiratsuka (2009) suggested that the lipid content was not affected by in puffer fish (*Takifugu ribripes*).

Fatty acid profile of puffer fish. Fatty acid composition from both liver and muscle of different puffer fish species are listed in Table 2 and 3. A total of 22 fatty acids comprising saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) were identified and the concentration was calculated based on the retention time. In general, the SFA content was higher in the liver compared to the muscle for all species. It was mainly composed of palmitic acid (C16:0) and stearic acid (C18:0) in all species studied. The result is in line with Hazra et al (1998) findings in Indian Coastal Waters' puffer fish (palmitic acid: 12.8%-34.6%) throughout the seasonal variations. The abundance of palmitic acid is not influenced by the diet despite being a key metabolite of fish (Bautista et al 1991), as indicated by the higher SFA values in the liver for all species studied. The highest SFA content is found in *L. cornuta* (35.43±1.18), followed closely by *X. naritus* (32.41±0.69) and *D. holocanthus* (32.5±0.58). *R. nasus* has the lowest SFA content among the species (31.52±1). The range of SFA from liver was 31.52-35.43%. Regarding the muscle, *X. naritus* has the highest SFA content (19.16±0.82), followed by *D. holocanthus* (17.93±0.76) and *L. cornuta* (18.37±0.33). *R. nasus* has the lowest SFA content among the species (16.67±0.32), with *D. hystrix* (16.73±0.25) having a slightly higher SFA content. The range of SFA from muscle was 16.67-19.16%.

The MUFA content was relatively similar between liver and muscle tissues across the species. There were no consistent patterns indicating a significant difference in MUFA content between the two tissue types. The main MUFA's in all species studied were palmitoleic (C16:1n-7) and oleic (C18:1n-9) acids. *X. naritus* has the highest MUFA content (37.87±1.08), followed by *L. cornuta* (34.83±0.58). *D. holocanthus* has the lowest MUFA content (28.47±0.12), while *D. hystrix* (30.84±0.77) and *R. nasus* (27.5±1.37) have a slightly lower MUFA content. The range of MUFA from liver was 27.5-37.87%. Regarding the muscle, *X. naritus* has the highest MUFA content (31±0.58), followed by *L. cornuta* (30.86±0.42) and *R. nasus* (29.83±0.48). *D. hystrix* has a low MUFA content among the studied species (25.36±0.37), while *D. holocanthus* has a slightly lower value (24.23±0.88). The range of MUFA in the muscle was 24.23-21%. The same trend was observed by Hazra et al (1998), namely that fatty acids in puffer fish livers were mostly saturated and monounsaturated (60%-70%).

The PUFA content was generally higher in the liver compared to the muscle for all species. *R. nasus* has the highest PUFA content (21.95±1.25) in liver, followed by *L. cornuta* (19.77±0.36) and *D. holocanthus* (19.18±0.97). *X. naritus* has the lowest PUFA content (14.63±0.16), while *D. hystrix* (16.65±0.08) have a slightly higher PUFA content. The range of PUFA from the liver was 14.63-21.95%. Hazra et al (1998) reported several different puffer fish livers containing a maximum of 21.1% of PUFA concentration. Regarding the muscle, *R. nasus* has the highest PUFA content (12.11±1.05), followed by *X. naritus* (10.81±0.26). *D. hystrix* has the lowest PUFA content (5.4±0.34), while *D. holocanthus* (8.07±0.39) and *L. cornuta* (7.97±0.6) have a slightly higher PUFA content. The range of PUFA from the muscle was 5.4-12.11%. The results are also comparable with catfish lipid level (12.11%) reported by Simplice et al (2018) in a previous study, tilapia lipid level (7.56%) reported by Suseno et al (2015), and salmo salar lipid level (10.64%) reported by Inguglia et al (2020). Koizumi & Hiratsuka (2009) reported that C18:0, C18:1n-9 and C18:2n-6 in Chinese cultured fish were lower than those in Japanese cultured fish. The composition of fatty acids is influenced by the diet, between Chinese and Japanese cultured puffer fish.

Table 2

Fatty acid composition of different puffer fish extracted from liver

Fatty acid	*X. naritus*	*D. hystrix*	*D. holocanthus*	*L. cornuta*	*R. nasus*
Myristic (C14:0)	2.49±0.09^{a}	2.95±0.05^{b}	2.51±0.03^{a}	2.95±0.03^{b}	3.09±0.02^{c}
Pentadecanoic (C15:0)	1.51±0.36^{a}	2.17±0.18^{b}	2.33±0.28^{b}	1.85±0.03^{a}	1.96±0.06^{a}
Palmitic (C16:0)	17.35±0.09^{a}	15.1±1.12^{b}	15.85±0.28^{b}	17.61±1.41^{a}	15.98±0.43^{b}
Heptadecanoic (C17:0)	2.05±0.91^{a}	3.05±0.11 ^{b}	3.08±0.09^{b}	2.72±0.02^{c}	2.39±0.31^{c}
Stearic (C18:0)	7.67±0.13^{a}	7.57±0.28^{a}	7.72±0.17^{a}	9.42±0.07^{b}	7.27±0.85^{c}
Heneicosanoic (C21:0)	0.77±0.1^{a}	0.78±0.07^{a}	0.75±0.02^{a}	0.52±0.03^{b}	0.57±0.12^{b}
Tricosanoic (C23:0)	0.23±0.06^{a}	0.14±0.05^{a}	0±0^{b}	0.14±0.05^{a}	0±0^{b}
Lignoceric (C24:0)	0.35±0.05^{a}	0.28±0.05^{b}	0.25±0.04^{b}	0.23±0.08^{b}	0.26±0.05^{b}
∑SFA	32.41±0.69^{a}	32.04±0.98^{a}	32.5±0.58^{a}	35.43±1.18^{b}	31.52±1^{ac}
Palmitoleic (C16:1n-7)	8.46±0.35^{a}	7.83±0.16^{b}	7.88±0.07^{b}	8.65±0.16^{a}	8.63±0.15^{a}
cis-10-heptadecenoic (C17:1n-7)	1.91±0.16^{a}	1.92±0.18^{a}	2.07±0.05^{a}	1.58±0.19^{b}	1.33±0.1^{b}
Vaccenic (C18:1n-7)	0.64±0.12^{a}	0.11±0.17^{a}	0±0^{b}	0±0^{b}	0±0^{b}
Oleic (C18:1n-9)	17.16±0.4^{a}	12.55±0.7^{b}	8.59±0.09^{c}	16.22±0.09^{a}	12.7±1.21^{b}
Paullinic (C20:1n-7)	1.27±0.07^{a}	0±0^{b}	0±0^{b}	0.74±0.2^{c}	1.24±0.1^{a}
Gondoic (C20:1n-9)	6.75±1.46^{a}	5.93±0.48^{b}	7.63±0.19^{c}	5.93±0.11^{b}	3.12±0.54^{d}
Erucic (C22:1n-9)	1.69±0.09^{a}	2.5±0.03^{b}	2.3±0.09^{b}	1.71±0.17^{a}	0.48±0.07^{c}
∑MUFA	37.87±1.08^{a}	30.84±0.77^{b}	28.47±0.12^{b}	34.83±0.58^{c}	27.5±1.37^{b}
Linoleic (C18:2n-6)	0±0^{a}	1.64±0.09^{b}	2.03±0.28^{c}	0±0^{a}	2±0.19^{b}
Arachidonic (C20:4n-6)	1.84±0.1^{a}	4.81±0.17^{b}	4.16±0.19^{b}	4.1±0.06^{b}	0.34±0.4^{c}
Adrenic (C22:4n-6)	1.01±0.1^{a}	2.14±0.09^{b}	0.18±0.03^{c}	4.09±0.31^{d}	1.55±0.13^{a}
DPA-6 (C22:5n-6)	1.18±0.05^{a}	1.36±0.09^{a}	1.7±0.16^{b}	0.44±0.63^{c}	1.85±0.09^{b}
EPA (C20:5n-3)	1.09±0.03^{a}	2.83±0.04^{c}	1.93±0.53^{b}	2.42±0.24^{c}	2.79±0.08^{c}
DPA-3 (C22:5n-3)	3.91±0.07^{a}	0.26±0.03^{b}	0.25±0.03^{b}	3.83±0.09^{a}	3.68±0.12^{a}
DHA (C22:6n-3)	5.59±0.09^{a}	3.61±0.21^{b}	8.93±1.15^{c}	4.9±0.69^{ab}	9.74±1.7^{c}
∑PUFA	14.63±0.16^{a}	16.65±0.08^{b}	19.18±0.97^{b}	19.77±0.36^{b}	21.95±1.25^{c}

Different superscripts in the same row within each fraction indicate significant differences at p<0.05.

Table 3

Fatty acid composition of different puffer fish extracted from muscle

Fatty acid	*X. naritus*	*D. hystrix*	*D. holocanthus*	*L. cornuta*	*R. nasus*
Myristic (C14:0)	0.76±0.15^{a}	0.89±0.03^{a}	0.76±0.13^{a}	1.13±0.19^{b}	2.64±0.55^{c}
Pentadecanoic (C15:0)	0±0	0±0	0±0	0±0	0±0
Palmitic (C16:0)	11.28±0.16^{a}	7.41±0.14^{b}	9.39±0.37^{c}	8.59±0.27^{c}	6.66±0.32^{b}
Heptadecanoic (C17:0)	1.06±0.12^{a}	3.44±0.04^{b}	2.6±0.19^{c}	1.8±0.13^{a}	1.66±0.09^{a}
Stearic (C18:0)	4.62±0.78^{a}	4.47±0.22^{a}	4.62±0.24^{a}	6.42±0.37^{b}	5.72±0.25^{c}
Heneicosanoic (C21:0)	1.43±0.11^{a}	0.53±0.05^{b}	0.55±0.2^{b}	0.42±0.11^{b}	0±0^{c}
Tricosanoic (C23:0)	0±0	0±0	0±0	0±0	0±0
Lignoceric (C24:0)	0±0	0±0	0±0	0±0	0±0
∑SFA	19.16±0.82^{a}	16.73±0.25^{b}	17.93±0.76^{abc}	18.37±0.33^{a}	16.67±0.32^{bd}
Palmitoleic (C16:1n-7)	7.86±0.31^{a}	6.56±0.21^{b}	6.39±0.4^{b}	7.59±0.34^{a}	7.52±0.18^{a}
cis-10-heptadecenoic (C17:1n-7)	1.54±0.1^{a}	1.41±0.17^{a}	1.5±0.18^{a}	1.07±0.11^{b}	1.13±0.11^{b}
Vaccenic (C18:1n-7)	0±0	0±0	0±0	0±0	0±0
Oleic (C18:1n-9)	18.94±0.56^{a}	13.34±0.18^{b}	13.14±0.75^{b}	19.24±0.24^{a}	17.31±0.21^{b}
Paullinic (C20:1n-7)	0±0	0±0	0±0	0.28±0.16	0.81±0.09
Gondoic (C20:1n-9)	2.66±0.27^{a}	2.7±0.16^{a}	1.65±0.26^{b}	1.52±0.4^{b}	2.57±0.19^{a}
Erucic (C22:1n-9)	0±0	1.34±0.08	1.55±0.29	1.14±0.19	0.49±0.43
∑MUFA	31±0.58^{a}	25.36±0.37^{b}	24.23±0.88^{c}	30.86±0.42^{a}	29.83±0.48^{ab}
Linoleic (C18:2n-6)	0±0^{a}	1.25±0.17^{b}	1.06±0.15^{b}	0±0^{a}	1.14±0.12^{b}
Arachidonic (C20:4n-6)	2.14±0.1^{a}	0.53±0.19^{b}	0.48±0.15^{b}	1.15±0.14^{c}	0.18±0.09^{b}
Adrenic (C22:4n-6)	0.25±0.07^{a}	1.04±0.16^{b}	0.17±0.11^{a}	1.5±0.16^{b}	1.05±0.17^{b}
DPA-6 (C22:5n-6)	0.69±0.08^{a}	0.48±0.08^{b}	0.39±0.11^{c}	0.38±0.12^{c}	0.27±0.11^{c}
EPA (C20:5n-3)	0.58±0.13^{a}	0.49±0.12^{a}	1.02±0.2^{b}	0.93±0.35^{cb}	0.83±0.13^{c}
DPA-3 (C22:5n-3)	3.39±0.14^{a}	0±0^{b}	0±0^{b}	1.56±0.37^{c}	1.58±0.35^{c}
DHA (C22:6n-3)	3.77±0.12^{a}	1.62±0.09^{b}	4.94±0.1^{a}	2.46±0.29^{ab}	7.06±0.74^{c}
∑PUFA	10.81±0.26^{a}	5.4±0.34^{b}	8.07±0.39^{c}	7.97±0.6^{c}	12.11±1.05^{a}

Different superscripts in the same row within each fraction indicate significant differences at p<0.05.

Quality index of puffer fish lipids. Table 4 shows several indices of fatty acid composition, providing an insight on the possible health effects of certain fatty acids. Significant differences were observed in PUFA/SFA levels in the livers of *X. naritus* and *R. nasus* while in the muscle, *X. naritus, D. hystrix* and *R. nasus* were significantly different from each other ($p<0.05$). The range of the w-6/w-3 ratio in the liver is from 0.36 to 1.49, while in the muscle, the range is from 0.28 to 1.57. The present study reported that *D. holocanthus, L. cornuta* and *R. nasus* showed significant differences in EPA+DHA content in the liver, compared to the other species ($p<0.05$). IA and IT indices of lipid extracted from the livers showed relatively low values compared to the muscle, in all species studied.

Table 4

Quality index of lipids from different puffer fish species

Quality indices	*X. naritus*	*D. hystrix*	*D. holocanthus*	*L. cornuta*	*R. nasus*
			Liver		
w-6/w-3	0.38±0.01^a	1.49±0.06^b	0.73±0.07^c	0.78±0.09^c	0.36±0.04^a
EPA+DHA	6.68±0.12^a	6.44±0.17^a	10.86±0.96^b	7.32±0.71^c	12.53±1.44^d
PUFA/SFA	0.45±0.01^a	0.52±0.02^b	0.59±0.02^b	0.56±0.01^b	0.7±0.02^c
IT	0.52±0.35^a	0.57±1.54^b	0.54±0.38^c	0.54±1.63^c	0.57±0.2^b
IA	0.5±0.24^a	0.62±0.77^b	0.49±0.09^a	0.53±0.34^a	0.39±0.12^c
			Muscle		
w-6/w-3	0.4±0.01^a	1.57±0.15^b	0.35±0.03^a	0.61±0.03^c	0.28±0.02^a
EPA+DHA	4.35±0.09^a	2.1±0.03^b	5.96±0.24^c	3.39±0.52^a	7.89±0.68^d
PUFA/SFA	0.57±0.04^a	0.32±0.02^b	0.45±0.01^c	0.43±0.03^c	0.73±0.06^d
IT	0.34±0.89^a	0.36±0.39^a	0.39±0.71^a	0.34±1.01^a	0.41±1.66^b
IA	0.39±0.52^a	0.6±0.72^b	0.44±0.31^a	0.48±0.22^a	0.31±0.11^c

Different superscripts in the same row within each fraction indicate significant differences at $p<0.05$.

The PUFA/SFA ratio is a commonly used measure to evaluate the impact of diet on cardiovascular health. PUFA in human diet can lower LDL cholesterol and overall serum cholesterol levels, while SFA contribute to higher cholesterol levels. Therefore, a higher PUFA/SFA ratio indicates a more beneficial effect on cardiovascular health (Chen & Liu 2020). PUFA/SFA ratio in the extracted fish lipid of the liver was observed in *R. nasus* (0.7±0.02) followed by *D. holocanthus* (0.59±0.02), *L. cornuta* (0.56±0.01), *D. hystrix* (0.52±0.02) and *X. naritus* (0.45±0.01). PUFA/SFA ratio of the muscle lipid was slightly lower in all species except *R. nasus* (0.73±0.06). For all species studied, the PUFA/SFA ratio in the liver was above the recommended minimum value of 0.4, except in the lipid extracted from the muscle of *D. hystrix* (Lukic et al 2021).

EPA+DHA is a widely recognized index found in dietary guidelines, recommending an intake of 0.250–2 g day^{-1}. The nutritional value of seafood is significant, particularly for fish, due to their high EPA and DHA contents (Chen & Liu 2020). The present study reported that highest EPA+DHA was found in lipids from both liver and muscle extracted from *R. nasus*. In general, EPA+DHA in liver was higher than in muscle, due to the higher amount of fatty acids content in the liver than in the muscle. The EPA+DHA levels are highly related to the level of fat, hence lean fish have relatively lower EPA+DHA levels as compared to other fatty fish species (Turan et al 2007). This imbalanced intake of omega-6/omega-3 could contribute to various diseases including cardiovascular issues, cancer, and inflammatory conditions. A lower omega-6/omega-3 ratio, achieved through an increased omega-3 intake, has been associated with positive effects in preventing and managing these diseases (Simopoulos 2002). Therefore, aiming for a lower omega-6/omega-3 ratio is beneficial in reducing the risk of chronic diseases prevalent in Western societies and worldwide. The present study indicates that there were variations in the ratio of omega-6 to omega-3 fatty acids between the liver and muscle samples of different species. The lowest ratios were found in the liver of *X. naritus* (0.38±0.01) and in the muscle of *R. nasus* (0.28±0.02), indicating a relatively higher proportion of omega-3 fatty acids compared to omega-6 fatty acids. On the other hand, the highest ratios were found in both liver (1.49±0.06) and muscle (1.57±0.15) of *D. hystrix*,

suggesting a higher proportion of omega-6 fatty acids compared to omega-3 fatty acids. *D. holocanthus* and *L. cornuta* have intermediate ratios of 0.73±0.07 and 0.78±0.09, respectively, in their liver, indicating a relatively balanced ratio between omega-6 and omega-3 fatty acids. A recommended ratio of omega-6/omega-3 fatty acids of 1:1 to 2:1 is supported by a research on the evolutionary diet, neurodevelopment, and genetics (Simopoulos 2010).

IA and IT, introduced by Ulbricht & Southgate in 1991, are measures that comparatively assess the atherogenic and thrombogenic properties of fatty acids. Lower IA and IT values indicate a higher nutritional value of the fatty acid, potentially reducing the risk of coronary heart diseases. In this study, IA and IT of all species from both liver and muscle were lower than 1, indicating positive health benefits. The puffer fish *Lagocephalus guentheri* was reported to have IA and IT of 0.43 and 0.29, respectively (Sreelakshmi et al 2019).

Physicohemical properties. The values of free fatty acid (FFA), acid value (AV), peroxide value (PV), iodine value (IV), and saponification value (SV) of puffer fish lipid obtained from livers and muscles are presented in Table 5. For livers, the FFA, AV, PV, IV and SV of the extracted lipid varied in the ranges 1.60±0.17-8.04±0.42 (%), 3.18±0.35-15.99±0.83 (mg KOH g^{-1}), 5.60±-0.43-10.67±0.34 (Meq kg^{-1}), 78.05±6.54-104.50±10.24 (g I_2 $100g^{-1}$) and 219.35±5.27-339.94±12.27 (mg KOH g^{-1}), respectively. Meanwhile, for muscles, the FFA, AV, PV, IV and SV of the extracted lipid varied in the ranges 1.55±0.11-2.96±0.11 (%), 3.09±0.23-5.89±0.23 (mg KOH g^{-1}), 4.47±0.81-9.47±0.68 (Meq kg^{-1}), 70.27±3.17-99.91±10.23 (g I_2 $100g^{-1}$) and 148.53±9.34-268.81±8.04 (mg KOH g^{-1}), respectively.

Table 5

Physicochemical properties of lipids from different puffer fish species

Physicochemical properties	*X. naritus*	*D. hystrix*	*D. holocanthus*	*L. cornuta*	*R. nasus*
Liver					
FFA (%)	1.60±0.17^{a}	3.24±0.64^{b}	3.05±0.24^{b}	8.04±0.42^{c}	5.92±0.11^{d}
AV (mg KOH g^{-1})	3.18±0.35^{a}	6.45±1.28^{b}	6.08±0.48^{b}	15.99±0.83^{c}	11.78±0.23^{d}
PV (Meq kg^{-1})	5.60±1.07^{a}	10.67±0.34^{b}	6.00±0.16^{a}	5.60±0.43^{a}	8.20±0.49^{c}
IV (g I_2 $100g^{-1}$)	80.43±15.29^{a}	104.50±10.24^{b}	97.38±12.69^{c}	81.28±9.50^{a}	78.05±6.54^{a}
SV (mg g^{-1} KOH)	311.82±3.04^{a}	240.85±16.93^{b}	273.11±10.97^{c}	219.35±5.27^{b}	339.94±12.27^{d}
Muscle					
FFA (%)	1.55±0.11^{a}	2.73±0.43^{b}	2.82±0.34^{b}	2.96±0.11^{b}	2.87±0.24$^{\mathbf{b}}$
AV (mg KOH g^{-1})	3.09±0.23^{a}	5.42±0.87^{b}	5.61±0.69^{b}	5.89±0.23$^{\mathbf{b}}$	5.70±0.48^{b}
PV (Meq kg^{-1})	4.47±0.81^{a}	9.47±0.68^{b}	5.07±0.47^{c}	4.53±0.34^{a}	7.53±0.38^{d}
IV (g I_2 $100g^{-1}$)	76.20±12.62^{a}	99.91±10.23^{b}	90.59±10.44^{b}	77.89±7.85^{a}	70.27±3.17^{a}
SV (mg g^{-1} KOH)	227.95±12.17^{a}	189.24±16.93^{b}	202.15±8.04$^{\mathbf{b}}$	148.53±9.34^{c}	268.81±8.04^{d}

Different superscripts in the same row within each fraction indicate significant differences at $p<0.05$.

The indication of hydrolytic rancidity, or the breakdown of fats in the presence of water, are FFA and AV. AV is a measurement of the quantity of acid needed to neutralise the free fatty acids in the oil, whereas FFA is a measure of the amount of free fatty acids present in the oil. A greater hydrolytic rancidity is indicated by an increased FFA and AV values. In both liver and muscle lipids, *X. naritus* had the lowest FFA and AV values in this instance, suggesting the least hydrolytic rancidity. On the other hand, *L. cornuta* showed the highest value of FFA and AV. This reveals that while all the species have free fatty acid levels within the recommended range (1-7%), their acid values are higher than the recommended limit (<3%) (Bimbo 1992). This might due to the hydrolysis of ester bonds in the presence of moisture and heat (Chantachum et al 2000). Acidity of oil is related to the presence of FFA and other non-lipid acid compound in which hydrolysis of triacylglyceride produces FFA, whereas the decay of feedstock produces a non-lipid acid

compound (Nazir et al 2017). An increasing free fatty acid is often directly proportional to an increase in the acid value (Barthet et al 2008). Unsaturated fatty acids react with oxygen to form peroxides, making PV a reliable indicator of oxidation. Lower values indicate better quality. PV monitoring is essential during processing and storage to ensure oil suitability (Nazir et al 2017). Both the American Society for Testing and Materials (ASTM) and the World Health Organization/Food and Agriculture Organization (WHO/FAO) have established a permissible maximum level of peroxide content in oils, which should not exceed 10 milliequivalents of peroxide oxygen kg^{-1}. This study reported that most oxidation occurred in the liver of *D. hystrix*, which has the highest PV value, whereby other species were within the recommended value. The iodine value is a measure of the degree of unsaturation of an oil or fat and indicates the amount of iodine absorbed by 100 g of the substance. An oil with a low iodine value has a greater ability to resist oxidation during storage. Conversely, an oil with a higher iodine value indicates a higher degree of unsaturation, which generally leads to better quality oil (Bako et al 2017). The most unsaturated fatty acids are present in *D. hystrix*, which has the highest IV value. A measurement of the average molecular weight of the fatty acids in the oil is the SV. A higher SV value indicates a higher molecular weight. *O. nasus* has the highest SV value, which represents that its fatty acid molecules have the highest molecular weight. All the species have saponification values higher than the recommended range of 179-206.2 mg KOH g^{-1} (Turchini et al 2010). However, the result was in line with Hazra et al (1998) who report, on four species of puffer fish (*L. lunaris*, *C. patoca*, *S. oblongus* and *L. inermis*), SV ranging from 230.6 to 458.5 mg KOH g^{-1}. This might be due to a high concentration of saturated short chain fatty acids, mainly palmitic acid (C16:0).

Conclusions. The findings of the current study provide evidence that puffer fish, often considered as low-value fish due to their tetrodotoxin content, have high levels of beneficial omega-3 fatty acids, including DHA and EPA, in their lipid composition. Moreover, the analyses of physicochemical and quality indices indicate that the fish lipid is safe and advantageous for human consumption.

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