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Effect of reproductive maturity on oil yield, proximate composition, and fatty acid profile of marine fish roe

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ABSTRACT

This study evaluated the effects of reproductive maturity on proximate composition, oil yield, and fatty acid profile of roe oil extracted from *Thunnus albacares, Katsuwonus pelamis, Canthidermis maculata, and Lepidocybium flavobrunneum*. Roe at different maturity stages (immature, maturing, and mature) were analyzed in triplicate. Proximate analysis was conducted to determine the lipid and other nutrient content. Roe oil was extracted using five techniques, namely heat hydrolysis, salt extraction, natural enzymatic hydrolysis, mechanical pressing, and solvent extraction. Fatty acid composition was analyzed using gas chromatography–mass spectrometry. The highest lipid content ($p < 0.05$) was observed in mature roe of all species compared to immature and maturing stages. Solvent extraction using isopropanol: hexane (2:1) yielded the highest ($p < 0.05$) roe oil. The highest oil recovery ($p < 0.05$) was obtained from mature *T. albacares* ($3.52 \pm 0.17\%$). Polyunsaturated fatty acids (PUFAs) predominated in roe oil with the highest levels detected in immature roe. The highest PUFA content ($38.29 \pm 1.04\%$) was observed in immature *T. albacares* roe oil. In contrast, *L. flavobrunneum* exhibited a significantly higher proportion of monounsaturated fatty acids, exceeding 45%. Results demonstrated that reproductive maturity and extraction techniques significantly influenced roe oil yield and fatty acid composition.

1. Introduction

Fisheries and aquaculture have experienced a boom due to global population growth and industrialization, which have been encouraged by fishing technologies of the highest caliber. Currently, fishery by-products and waste have increased significantly as a result, with approximately half of the catch for human consumption being discarded (Mgbechidinma et al., 2023). Heads, skin, viscera, and frames are main fishery by-products often overlooked and underutilized, yet abundant in essential nutrients such as lipids, proteins and functional bioactive compounds including polyunsaturated fatty acids (PUFAs), vitamins and collagen, as well as industrially important compounds such as chitin, gelatine, pigments, and various enzymes (Rohim et al., 2024; Roy et al., 2023). Currently, fish wastes, and by-products are partially utilized to produce fishmeal, fertilizers, and fish oil, often yielding low profitability (Zhang et al., 2021). Fish oil extracted from fish waste is directly used as one of the lipid sources to produce animal feeds in aquaculture, poultry, and swine industries (Hossain et al., 2023). It is believed that fish oil is an excellent source of PUFAs essential to human health, including omega-3 fatty acids like cis-5,8,11,14,17-Eicosapentaenoic acid (EPA) and cis-4,7,10,13,16,19-Docosahexaenoic acid (DHA), which have proven preventive effects on cardiovascular diseases, neurological disorders and cancer while also playing a crucial role in infant brain and vision development and enhancing immunity (Chen et al., 2022). The oil content of fish by-products can be significantly varied from 1.40% to 40.10%, influenced by factors such as species, tissue type, nutritional status, season, maturity stages and environmental conditions (Mgbechidinma et al., 2023). The projection suggests that fish oil for human consumption will reach 771,000 tons by 2025 (Alfio et al., 2021). This increasing demand emphasizes the need for alternative sources, such as fish roe, rather than solely relying on traditional sources like wild and farmed fish (Guedes et al., 2020). Among these by-products, fish roe, found within the fish's ovarian sac, is often disregarded in fish oil production despite its nutritional value. However, it's a major by-product in global fisheries, with growing demand for caviar production (Guedes et al., 2020). While some limited types, like

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sturgeon roe, are popular as caviar, most fish gonads are ignored due to their low market value. Unfortunately, most of the fish roe is often discarded or processed into low-value fish meals instead of being used as nutritious seafood (Furey et al., 2020; Tsamesidis et al., 2023). Fish roe, rich in lipids (2.5–17.8%), contains various types such as phospholipids, glycolipids, triacylglycerols and wax esters. Notably, it is abundant in EPA, DHA, and saturated fatty acids (SFA) (Guedes et al., 2020). Moreover, fish roe lipids possess diverse benefits, including inhibiting colon cancer cell growth and providing antioxidant and immunostimulant properties. Furthermore, fish roe exhibits promising potential for incorporation into functional foods, nutraceuticals, and pharmaceutical formulations (Guedes et al., 2022). The extraction process and intrinsic parameters such as fish species, age, reproductive status, and seasonal fluctuations significantly influence the physicochemical qualities, fatty acid composition, and yield of fish roe oil (Georgiou et al., 2023; Tommonaro et al., 2023). Despite emerging interest in utilizing fish by-products, studies focusing on roe oil extraction remain limited, particularly with respect to variations across maturity stages and various extraction techniques (Georgiou et al., 2023; Luksta et al., 2024).

In Sri Lanka, fish roe is largely generated from marine fisheries and is often considered a low-value by-product and is either sold at low value or discarded because of limited consumer demand and the lack of value-added roe-based products such as caviar (Jayamanne et al., 2015). This is particularly evident in the tuna processing industry, where commercially valuable fish such as yellowfin and skipjack tuna are processed for export, while viscera, belly, flaps, and gonads are underutilized or wasted (Jayamanne et al., 2015). In addition, roe of underutilized species such as trigger fish (*Canthidermis maculata*) and escolar fish (*Lepidocybium flavobrunneum*) is also discarded without any value addition. Since the nutrients such as lipids, and fatty acids change with reproductive maturity, understanding these changes is advantageous for effective utilization. However, such specific studies examining both compositional variations and oil extraction efficiency across different extraction methods remain limited in the existing literature.

Therefore, this study aimed to evaluate the effect of reproductive maturity on nutrient composition, oil yield, and fatty acid profile of selected underutilized marine fish roe. In addition, the study uniquely evaluated roe from multiple fish species across various reproductive maturity stages, including immature, maturing and matured stages, and compared five extraction techniques such as mechanical pressing, heat and enzymatic hydrolysis, salt-assisted, and solvent extraction techniques within a single study to determine their influence on roe oil recovery. Furthermore, this study represents the first comprehensive evaluation of compositional changes of C. *maculata* and *L.* flavobrunneum roe with respect to the maturation process, delivering new insights into their potential as sources of nutritionally valuable oils.

2. Materials and methods

2.1. Sample selection and collection

Ovary samples of female *Thunnus albacares* (Yellowfin tuna-TA), *Katsuwonus pelamis* (Skipjack tuna-KP), *Canthidermis maculata* (Rough triggerfish-CM), and *Lepidocybium flavobrunneum* (Escolar fish-LF) at different maturity levels were collected from the local fishermen's landing site in Negombo fishing harbour. Samples were stored in the freezer at −18 ℃ until further use at the Aquaculture Laboratory of Uva Wellassa University, Badulla. To ascertain the maturity stage, a 4 ± 0.5 cm cross-section taken from the middle part of the bigger ovary lobe was detached and kept in buffered 4% formaldehyde (neutral) for histological examination. All the solvents and chemicals used were purchased from Sigma-Aldrich Ltd. (St. Louis, MO, USA) and Merck Specialties Pvt. Ltd. (Mumbai, India) were of analytical grade.

2.2. Determination of maturity level

The maturity of ovaries was determined by observing the external morphological characteristics and further confirmed by examining the internal characteristics using a biological microscope (MB.1052; Euromex, *Arnhem, Netherlands*) through an S40 × objective lens (Brown-Peterson et al., 2011). The average roe diameters were measured for each ovary to compare with previous studies, and they were categorized into three levels: immature (M1), maturing (M2), and mature (M3) (Batts, 2011; Branco et al., 2013; Grande et al., 2012; Zudaire et al., 2014). Composite roe samples were prepared by combining roes ($n = 5$) and all the analyses were conducted as replicates.

2.3. Proximate composition analysis of roe

The proximate compositions of the roe including moisture, crude lipid, protein, fiber, and ash, contents were analyzed in triplicate in each maturity level according to AOAC (2005) standard methods. Crude protein was determined by the Kjeldahl method, while moisture and ash contents were calculated using oven drying at 105 °C and muffle furnace incineration at 550 °C, respectively. Fiber content was analyzed using a fiber analyzer (FIWE Advance, VELP Scientifica Srl, Italy).

2.4. Extraction of oil from fish roe

The roe samples from each species with each maturity level were used to extract roe oils. The Roe sample was pretreated by homogenization at 10,000 rpm for 15 min (OV5, VELP, Italy) followed by sonication using a water bath sonicator at 30 ℃ for 30 min (SONER 206, Rocker Scientific Co., Ltd. Taiwan). Pretreated samples were subjected to five different methods of oil extraction, namely heat hydrolysis (HH), mechanical pressing extraction (ME), natural enzymatic hydrolysis (NEH), salt extraction (SE), and solvent extraction (SolE) as described by Deepika et al. (2014) with slight modifications. For mechanical pressing, the 25 g of pretreated roe samples were first transferred to a wire mesh and steamed for 30 min at 95 ℃. Subsequently the steamed samples were manually pressed using a hand-operating screw press machine. The roe oil was separated by centrifuging the extracted liquid at 20,000 g for 20 min at 4 ℃ using a centrifuge (Sorvall Biofuge Prime R, Thermo Scientific, USA). In heat hydrolysis, 100 mL of distilled water was added to 25 g of pretreated sample and the mixture was stirred at 5000 rpm for 30 min continuously at 90 ℃. Then the heat-treated mixture was centrifuged at 20,000 g for 20 min at 4 ℃ to recover the extracted oil layer. For salt extraction, 100 mL of NaCl solution (5% w/v) was added to the 25 g of pre-treated roe sample (NaCl:Roe=4:1), followed by stirring at 5000 rpm for 30 min at 55 ℃ and then treated in a water bath (YCW-010E; Gemmy Industrial Corp., Taipei, Taiwan) at 50 ℃ for 180 min. The extracted oil was separated by centrifugation under same conditions as previously mentioned. To conduct natural enzymatic hydrolysis, 25 mL of distilled water and 50 mL of a filtered mixture of ground fish viscera and crab carapace juice were added to 25 g of pretreated roe. pH was adjusted to 7 and the final mixture was continuously stirred at 40 ℃ for 240 min. The mixture was heated at 90 ℃ for 10 min to terminate the reaction, and oil was recovered by centrifugation as previously mentioned. Solvent extraction was carried out according to Iberahim and Tan (2020) with slight modifications using different solvents such as isopropanol (99.7% v/v), hexane (95% v/v), acetone (99.5% v/v), and the isopropanol:hexane (2:1) (v/v). Roe samples (25 g) were extracted using a solvent-to-sample ratio of 4:1 (v/w), corresponding to 100 mL of solvent per extraction, to find out variation in oil yields among the solvents. Briefly, the solvent was added to pretreated roe and the mixture was stirred at 40 °C for 240 min to enhance lipid extraction. The resulting mixture was filtered using a Whatman No. 1 filter and the solvent phase containing the extracted oil was separated. Roe oil was recovered by evaporating solvent using a rotary evaporator

(R-100, BUCHI Corporation, USA) at 40 °C under low pressure (45 mbar). All extracted oils were stored in sealed containers after flushing under nitrogen to prevent oxidation until further analysis.

2.5. Determination of fish roe oil yield

Weights and volumes of oils extracted from each method for each species were measured and recorded. The yield was calculated using equation 01 (Liu et al., 2021).

$$\text{Oil yield}(\%) = (WO/WR) \quad \times 100 \tag{1}$$

Wo is weight of the extracted oil (g) and W*R* is the weight of wet raw fish roe (g).

The extraction process that resulted the highest yield of roe oil was determined to be the best extraction method for extracting fish oil from the roe.

2.6. Determination of fish roe oil yield with the roe maturity level

The oil yield from each maturity level (M1, M2, M3) was calculated to observe the variation of roe oil yield.

2.7. Determination of fatty acid composition profile

Fatty acid methyl esters prepared in accordance with the Association of Analytical Communities method (AOAC, 2005) were used to determine the fatty acid compositions of roe oil extracted from each maturation stage of all the species. Gas chromatography (7890B, Agilent, USA) was used, along with an autosampler (7693, Agilent, USA), and a mass spectrometer (5977 A, Agilent, USA). Separation was conducted using a 100 m capillary column (TR-FAME 100 m × 0.25 mm ID, 0.20 μm film) (Thermo Scientific, Australia). Helium was used as the carrier gas and maintained at a constant pressure flow rate of 451.6 kPa with a split ratio of 25:1. The inlet and detector temperatures were 240 °C and 250 °C, respectively. The temperature program was initiated with an initial temperature of 150°C and held for 5 min. Then it increased to 200 °C at 2 °C/min with a 5 min hold, then increased to 210 °C at 4 °C/min with a 5 min hold, and finally increased to 240°C at a 6 °C/min rate and kept for 10 min (Shanuke et al., 2025). All the analyses were performed in triplicate and standard mass spectrometry was used to identify the fatty acids in fish roe oils using a fish oil standard (Supelco 37 Component FAME Mix, Sigma-Aldrich Co. Ltd., USA). Fatty acids were identified by comparing retention times and mass spectra with those of a standard FAME mixture (Supelco 37 Component FAME Mix, Sigma-Aldrich, USA). Quantification was performed by area normalization, and the results were expressed as the relative percentage of each fatty acid based on total peak area.

2.8. Estimation of oxidation parameters of fish roe oil

To investigate the quality of roe oil, the estimation of peroxide value (PV) and acid value (AV) of extracted roe oil was analyzed according to the standard method of AOCS (1990).

2.9. Statistical analysis

All the experiments were conducted in triplicate and reported as means ± standard deviation. Analysis of variance (ANOVA) tests were used for statistical analysis of the results at the 95% level ($p < 0.05$) of significance. Also, the Tukey test was applied to determine significant differences. To perform statistical analysis tests, Minitab software version 19 (Minitab Inc., PA, U.S.A.) was used.

3. Results and discussion

3.1. Proximate composition analysis of fish roe

Table 1 demonstrates the proximate composition of roe at various stages of maturity. The proximate composition provides insight into the nutritional values, while the crude lipid content reveals the potential of oil extraction from the roe. Across all species, the gonadal maturation process from M1 to M3 led to a significant increase ($p < 0.05$) in crude lipid content, with mature roe demonstrating the highest lipid levels. In particular, mature roe of *L. flavobrunneum* exhibited the highest crude lipid percentage compared to other species. Ash content did not differ significantly ($p > 0.05$) among species or maturity stages, except in *K. pelamis*. Moisture content decreased progressively from immature to mature stages in all species. These results indicate that reproductive maturity significantly influences the proximate composition of roe. LF consistently exhibited the highest lipid content ($p < 0.05$) across all maturation stages compared to other species. However, it's noteworthy that more than 90% of the total oil content in LF roe comprises indigestible wax esters (Pattaravivat et al., 2008). The proximate composition of *T. albacares*, *K. pelamis*, and *C. maculata* are consistent with previous studies (Intarasirisawat et al., 2011; Lee et al., 2016; Williams and Munasinghe, 2018; Yoon et al., 2018).

Fish roe's chemical composition is greatly influenced by maturity stage, food, season, and living environment. Usually, roe diameter, yolk globules and oil globules in fish eggs are developed by size and number during the vitellogenesis and secondary development stages (Brown-Peterson et al., 2011). As a result of this maturation process, matured roe at exhibited the highest lipid content. This is associated with endocrine-regulated lipid mobilization and selective deposition during oogenesis (Hayashida et al., 2025). Generally, high oestrogen level in female fish stimulates hepatic synthesis of vitellogenin and very low-density lipoproteins, which transport lipids to developing oocytes. In addition, increasing lipolytic activity also releases stored lipids into circulation. These released lipids are up taken by oocytes via receptor-mediated endocytosis and utilized for membrane formation and accumulated as oil droplets (Hayashida et al., 2025). Enzymatic activities, including lipoprotein lipase and fatty acid synthase, further enhance lipid deposition, leading to progressive lipid enrichment in mature roe. This metabolic and hormonal functions responsible for progressive lipid accumulation in mature roe (Dhurmeea et al., 2016).

For instance, during reproductive maturation of female Pacific bluefin tuna (*Thunnus orientalis*), lipids are transferred from muscle, liver, and perigonadal fat to the ovaries, resulting in increased lipid accumulation in roe whereas, roe lipid composition was dominated by phospholipids and triacylglycerols, while muscle lipid content remained comparatively low (Hayashida et al., 2025). Similar trend was reported by Dhurmeea et al. (2016), which demonstrated that lipids in somatic tissues of female albacore tuna (*Thunnus alalunga*), have been reallocated to the gonads during the maturation process, resulting in the highest total lipid accumulation in roe.

3.2. Determination of fish roe oil yields with different solvents

In the preliminary stage, the most effective solvent for roe oil extraction was identified based on maximum oil recovery. Matured roe was used for the oil extraction since it contained the highest crude lipid content as observed by the proximate composition analysis.

The variations in oil yields from each species depend on the extracted solvent, as displayed in Fig. 1. According to the results, the isopropanol: hexane mixed solvent reported the highest oil yields for KP, CM, and LF while acetone extraction resulted in the lowest oil yields across all roe samples. When compared to the other three roe types, CM roe produced the lowest oil content out of all the solvents that were examined. As depicted by Fig. 1, roe oil yield varied with the extraction solvents. Roe oil yields changed significantly ($p > 0.05$) among the fish species due to

Table 1
Changes in the proximate compositions of different roes along with maturation.

Parameter	Maturity Stage	*Thunnus albacares*	*Katsuwonus pelamis*	*Canthidermis maculata*	*Lepidocybium flavobrunneum*
Moisture	M1	74.92 ± 1.53^{a}	73.43 ± 1.91^{ab}	75.36 ± 1.50^{a}	73.29 ± 1.76^{ab}
	M2	74.30 ± 0.63^{a}	73.51 ± 1.70^{ab}	74.65 ± 0.55^{a}	69.35 ± 1.35^{bc}
	M3	72.59 ± 0.50^{ab}	71.66 ± 0.61^{b}	73.48 ± 1.37^{ab}	66.87 ± 2.90^{c}
Protein	M1	20.54 ± 1.66^{a}	21.40 ± 0.63^{a}	20.10 ± 0.94^{a}	15.99 ± 0.70^{bc}
	M2	20.16 ± 1.04^{a}	20.46 ± 1.84^{a}	19.29 ± 0.63^{ab}	15.02 ± 1.72^{c}
	M3	20.33 ± 0.22^{a}	20.92 ± 0.21^{a}	19.54 ± 0.80^{a}	14.77 ± 1.34^{c}
Lipid	M1	0.97 ± 0.09^{ef}	0.78 ± 0.16^{f}	0.42 ± 0.09^{f}	2.78 ± 0.59^{cd}
	M2	2.02 ± 0.12^{def}	1.65 ± 0.18^{def}	1.15 ± 0.05^{ef}	9.3 ± 1.11^{b}
	M3	3.78 ± 0.25^{c}	3.21 ± 0.11^{cd}	2.43 ± 0.20^{cde}	12.64 ± 1.34^{a}
Ash	M1	1.12 ± 0.01^{b}	1.06 ± 0.06^{b}	1.15 ± 0.07^{b}	1.01 ± 0.10^{b}
	M2	1.24 ± 0.20^{b}	1.23 ± 0.17^{b}	1.08 ± 0.08^{b}	0.99 ± 0.02^{b}
	M3	1.25 ± 0.18^{b}	1.76 ± 0.09^{a}	1.17 ± 0.13^{b}	1.01 ± 0.03^{b}

M1: immature roe, M2: maturing roe, M3: matured roe. Means with different superscript letters within each proximate parameter differ significantly ($p < 0.05$).

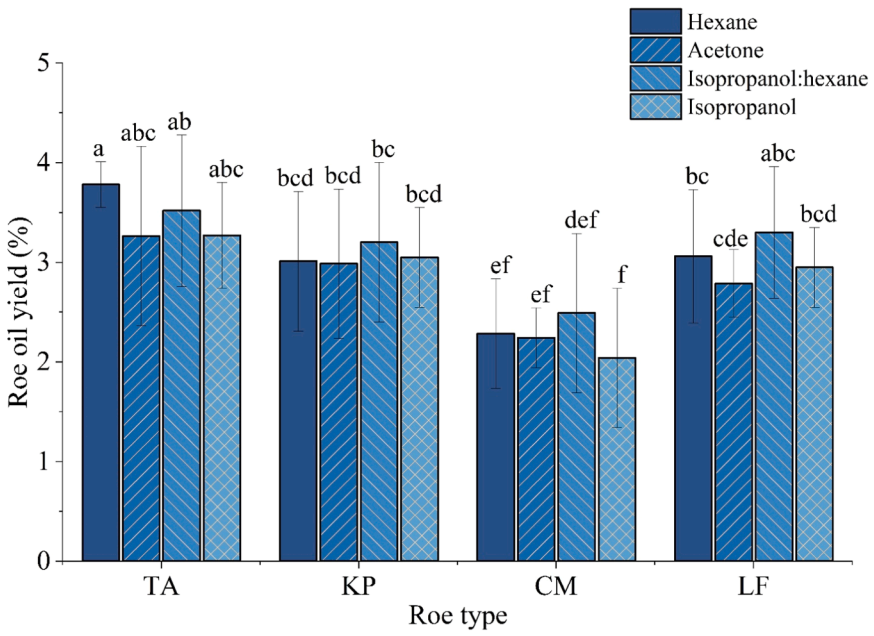

Fig. 1. The roe oil yield extracted with different solvents. TA: *T. albacares*, KP: *K. pelamis*, CM: *C. maculata*, LF: *L. flavobrunneum*. Different lowercase letters indicate significant differences among roe oil yields ($p < 0.05$; two-way ANOVA, Tukey's post hoc test).

species-specific physiological characteristics and reproductive metabolism, which alter the lipid accumulation patterns and composition of the roe (Tommonaro et al., 2023).

The highest roe oil yield from *T. albacares* was observed by hexane extraction, as the roe of *T. albacares* contained a higher proportion of neutral lipids compared with polar lipids (Zudaire et al., 2014). The neutral lipids in the roe of TA constitute more than 60% of the total lipids in mature ovaries, whereas the non-polar fatty acid-based wax esters account for 90% of the total lipids in LP (Đorđević et al., 2016). In contrast, mature roe of KP contains a high proportion of polar phospholipids, followed by sterols, wax esters, and triglycerides, which are composed of neutral and low-polar lipids. Therefore, the highest roe oil yield was extracted from using the isopropanol-hexane solvent mixture (Grande et al., 2012). Similarly, the highest roe yield from CM was observed by the isopropanol-hexane solvent mixture. The effectiveness of solvent extraction depends on lipid solubility, solvent polarity, and the ability to disrupt lipid-matrix interactions. Polarity is the primary factor that determines the effectiveness of solvent extraction. Non-polar lipids such as triacylglycerols and wax esters are highly soluble in non-polar solvents like hexane, whereas polar lipids such as phospholipids are soluble in polar solvents as polar solvents can break lipid-protein and membrane interactions, thereby facilitating the extraction process (Yin et al., 2018). Hexane is an affordable, non-polar solvent widely used for lipid extraction due to its high solubility for neutral lipids such as triacylglycerols at relatively low temperatures and it has low reactivity towards oil, and processing equipment. However, its extraction efficiency is limited for polar lipids such as phospholipids, which have greater affinity for polar solvents (Iberahim and Tan, 2020).

Most of the neutral and polar lipids in fish roe are structurally associated with proteins and cellular membranes, limiting their extractability. Organic solvents can disrupt lipid-protein and lipid-membrane interactions, thereby facilitating the release of bound lipids (Rohim et al., 2024). Combined solvent systems such as isopropanol: hexane can improve extraction efficiency by a synergistic effect. Isopropanol, as a polar solvent has the ability to destroy lipid-protein complexes and increases membrane permeability and extract polar lipids, while hexane efficiently solubilizes released neutral lipids. This complementary function enhances the overall oil recovery by this simultaneous extraction of both polar and non-polar lipids in fish roe (Iberahim and Tan, 2020). In addition, mixed solvents improve mass transfer by reducing interfacial tension and enhance solvent diffusion within the tissue matrix (Tsamesidis et al., 2023). Overall, combined solvent systems of isopropanol and hexane demonstrated higher extraction efficiency compared to other solvents and provided an effective balance between polarity and solvation ability, resulting in improved roe oil extraction across different species.

3.3. Determination of fish roe oil yields with different extraction methods

Fig. 2 illustrates the roe oil yields obtained by various extraction methods. In comparison to the other extraction methods studied, solvent extraction produced the considerably greatest levels of roe oil ($p < 0.05$) across all four types of roe Iberahim and Tan (2020) have also demonstrated solvent extraction's efficacy in extracting high-quality fish oil, particularly omega-3-rich oil from Atlantic salmon, using a hexane and isopropanol solvent system. Current findings emphasize solvent extraction's suitability for extracting oil from fish roe. The salt extraction yielded the lowest roe oil yields of all roe types except LF. Inorganic salt like NaCl increases the accessibility of oil for extraction and improves the efficiency of essential oil extraction by promoting mass and heat transfer, which breaks down cellular components (Suryanti et al., 2023). Since the addition of NaCl to the oil extraction process was effective in practical applications, it was expected that using NaCl at high temperatures would enhance oil accessibility in fish roe extraction. However, this method was ineffective for fish roe oil extraction.

Crab carapace extract and fish viscera extract were selected as natural enzyme sources due to their low cost, local availability, and potential for sustainable utilization of seafood processing waste (Nargotra et al., 2024). Fish viscera contain endogenous digestive enzymes, including proteases and lipases, while crab-derived extracts provide proteolytic and chitinolytic enzymes, enabling partial disruption of lipid-protein complexes and membrane-associated phospholipids (Caruso et al., 2020). This broad enzymatic function can facilitate limited hydrolysis of structural components within the roe matrix. Enzymes such as fish pepsins and collagenolytic enzymes from crab hepatopancreas have been widely utilized in industrial fish roe processing, such as enzymatic caviar production due to their ability to hydrolyse connective tissues and protein matrices (Caruso et al., 2020). Therefore, crab carapace and fish viscera-derived enzymes were utilized in this study as a natural, low-cost, and sustainable alternative to commercial enzymes for facilitating lipid release from roe to facilitate the roe oil extraction process. Furthermore, these fish enzymes exhibit high stability over a wide pH range and high temperature conditions compared to synthetic enzymes (Ismail and Hassan, 2022). Although less efficient than purified enzymes, these natural systems operate under mild conditions, which may reduce lipid oxidation and preserve

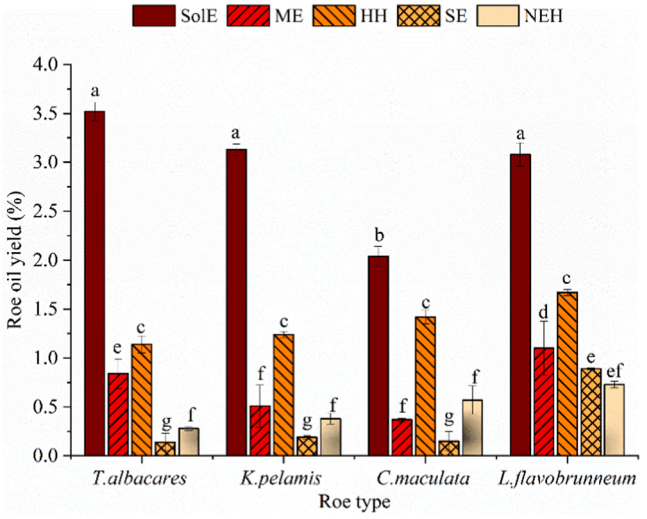

Fig. 2. Roe oil yields obtained from different extraction methods for each roe species. SolE: solvent extraction, ME: mechanical extraction, HH: heat hydrolysis, SE: Salt extraction and NEH: natural enzymatic hydrolysis. Different lowercase letters within each roe type indicate significant differences ($p < 0.05$). Different lowercase letters within each roe type indicate significant differences among roe oil yields ($p < 0.05$; two-way ANOVA followed by Tukey's post hoc test).

polyunsaturated fatty acids. However, the absence of controlled enzymatic activity and limited hydrolytic efficiency restrict complete breakdown of cellular structures and lipoprotein complexes, resulting in lower oil release.

Furthermore, there were no notable roe oil yield from natural enzymatic hydrolysis, which may have been caused by the natural enzymes' reduced efficiency and purity when contrasted with refined commercial proteolytic enzymes. These commercial enzymes are more effectively suited for hydrolysing the protein matrix and releasing bound lipids (Gbogouri et al., 2006; Zhang et al., 2021). Previous studies have reported that enzymatic hydrolysis using commercial proteases obtained by bacterial protease HT has improved lipid extraction from hoki roe compared to fungal protease FP-II by effectively degrading protein matrices (Ahmmed et al., 2022). In contrast, the present study observed low oil yield from natural enzymatic hydrolysis, likely due to lower enzyme activity and specificity. This indicates that, although natural enzyme sources can partially disrupt lipid-protein interactions, they are less efficient than purified commercial enzymes in releasing bound lipids.

Mechanical extraction is recognized as a convenient and safe method for extracting oil from fish muscle due to thermal denaturation of myofibrillar proteins, which disrupts fibrous structures and facilitates oil release (Alfio et al., 2021). This is primarily due to the cooking process, typically conducted at temperatures ranging from 90 to 115 °C, which effectively denatures the protein structure of fish muscle and enhances the oil-releasing capability of tissues. However, the lower roe oil yield obtained can be attributed to the specific microstructure of roe. Fish roe consists of intact oocytes in which lipids are compartmentalized within yolk granules, and oil droplets are associated with lipoprotein complexes and enclosed by membrane systems (Rohim et al., 2024). This structure requires higher mechanical and thermal energy for disruption. Fish muscle tissue has a fibrous structure that efficiently transmits mechanical pressure. Fish cell membranes are composed of phospholipid bilayers, which act as barriers to lipid extraction. Disruption of membrane integrity through pore formation and structural damage facilitates the release of bound oils (Rohim et al., 2024). In contrast, roe is composed of discrete, spherical cells that deform rather than rupture under mechanical force, thereby limiting oil release. However, the lower yield was observed in the present study, indicating that the moderate operating conditions, such as heating temperature, time, and low mechanical pressure, may be insufficient for extracting oil from fish roe. Accordingly, the structurally protected and compartmentalized nature of roe lipids has reduced the efficiency of mechanical pressing.

Heat hydrolysis of fish roe also did not extract a significantly high oil yield in this study. Applying optimum heat and time duration are critical to obtaining a higher fish oil yield. According to Jayasinghe et al. (2013), many fish meal plants employ cooking temperatures above 90 °C, yet the range of 77–80 °C has demonstrated the highest oil recovery percentage from heat hydrolysis. Rubio-Rodríguez et al. (2010) emphasized the importance of appropriate heat treatment in achieving a high fish oil yield. The operational conditions employed in the heat hydrolysis method involved heating at 90 °C for 30 min, resulting in a higher roe oil yield compared to other methods. It served as the second effective extraction method, yielding substantial roe oil following solvent extraction. Although the yield of oil is slightly higher, the overall yields remained unsatisfactory. This may be attributed to the higher temperature applied which caused lipids to trap inside the packed unfolded proteins, thereby reducing the oil yields from heat hydrolysis (Gbogouri et al., 2006). Even though LF roe had the highest crude lipid content, the yield of oil extracted from LF roe did not exhibit a significant difference compared to other roe varieties. The existence of an abnormally large percentage of wax esters, which are made up of neutral fatty acids and do not dissolve well in polar solvents, is the reason for the reduced extraction efficiency even with its greater crude lipid content (Pattaravivat et al., 2008).

Georgiou et al. (2023) studied the effect of different extraction

techniques, including solvent extraction, supercritical fluid extraction, expeller oil press, expeller oil press combined with solvent extraction, and wet reduction on mullet roe oil yield and quality. This study reported that extraction methods have significantly influenced oil yield and quality. The solvent-based methods have outperformed mechanical and enzymatic techniques. In particular, expeller oil press combined with solvent extraction has yielded 76% and 65% of roe oil, respectively. Similar to the current study, expeller oil press and wet reduction methods yielded the lowest roe oil yields, highlighting the importance of optimized processing conditions specifically for roe oil extraction. Kalogianni et al. (2023) compared the efficiency of different oil extraction techniques on mullet roe oil extraction, and the reported results are consistent with the present study, where solvent extraction gave the highest oil yield, followed by supercritical fluid extraction and pressure extraction (68%, 28%, and 10%, respectively). These findings support the present results, where solvent extraction, especially using mixed solvents, resulted in higher roe oil yields compared to other extraction methods. The modern technique such as supercritical CO_2 extraction was applied to extract roe oil from rainbow trout, and African catfish by Luksta et al. (2024), and reported that using supercritical CO_2 with ethanol as a co-solvent is more suitable for roe oil extraction. Tsamesidis et al. (2023) have referred to various oil extraction techniques such as mechanical pressing, solvent extraction, and use of enzymes like alcalase and protease for roe oil recovery. Although the processing conditions differed slightly from the present study, their findings are comparable, demonstrating that roe oil yield and quality were significantly determined by the extraction method.

3.4. Determination of fish roe oil yield with the roe maturity level

An isopropanol: hexane (2:1) solvent system was used to extract oil from roe at various maturity levels. As illustrated in Fig. 3, the roe oil yield exhibited a significant increase ($p < 0.05$) from M1 to M3 across all roe samples, with the highest roe oil yields achieved by matured samples from all four roe types. The trend of lipid accumulation observed in the present study highlights the importance of lipids in the roe maturation process. Lipids are the main metabolic energy source in reproduction, and body lipids are mobilized to ovaries mainly as polar lipids when the fish reach sexual maturity. Hence, the size and number of oil globules increase from immature to mature stages. Most of the time, immature roe does not contain a considerable number of oil globules except for much smaller oil globules found occasionally in some immature roe (Grande et al., 2012). Therefore, mature ovaries are a better option for use in the roe oil extraction process.

Changes of lipid and protein content are also related to the spawning environment of fish. Fish like salmon and trout, who spawn in nutrient-poor environments, tend to form roe with higher lipid and protein content. These higher accumulations in developing roe ensure the sufficient energy reserves for embryonic and early larval development. In contrast, many marine species produce eggs with lower lipid and protein accumulations since their spawning environments supply relatively accessible food sources for larvae. This ecological adaptation may also be another factor to explain the observed variations in roe oil content and composition across different species and maturity stages (Tsamesidis et al., 2023).

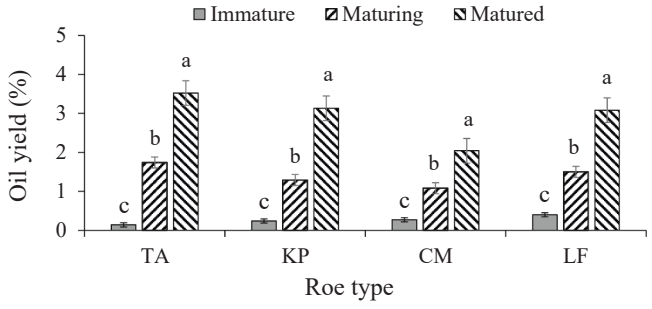

Fig. 3. Variation of extracted roe oil yield with maturity level of roe type. TA: *T. albacares*, KP: *K. pelamis*, CM: *C. maculata*, LF: *L. flavobrunneum* with different letters differ significantly ($p < 0.05$). Different lowercase letters within each roe type differ significantly ($p < 0.05$).

3.5. Determination of the fatty acid composition of roe oil extracted from different maturity stages of roe

The variation in fatty acid composition of the ovaries among the four selected fish species throughout maturation is shown in Fig. 4. Palmitic acid (C 16:0) was the predominant (more than 50%) fatty acid of total saturated fatty acids (SFA) in the gonads, followed by stearic acid (C 18:0) (more than 20%). Palmitic acid serves as a main energy source for fish metabolism during maturation, particularly during roe formation in females (Phetchthumrongchai et al., 2022). Since palmitic acid is the main contributor for SFA profile, the proportion of total SFA in gonads did not change significantly ($p > 0.05$) in TA or CM during maturation. The highest total SFA content was recorded by the roe of LF compared to other species.

As depicted in graph 4d, LF roe in the three development stages had the highest content of monounsaturated fatty acids (MUFA) compared to other fish species. However, the MUFA content of LF varied significantly during the maturation process, unlike the other three species. A significant portion of the total MUFA content in all roe has been provided by oleic acid, which comprises more than 75% of the total MUFA (C18:1 n-9). Significantly higher content of oleic acid percentages ranging from 36.85 ± 1.54 (M1) to 33.14 ± 2.89 (M3) was observed in LF due to the presence of predominant wax esters, primarily composed of MUSFs (Nichols et al., 2001). Oleic acid is the main MUFA, which supports metabolic processes by providing energy for reproductive organ development and plays a crucial role in ovarian development and overall fish growth (Phetchthumrongchai et al., 2022).

The polyunsaturated fatty acid (PUFA) content of roe at each maturity stage of TA, KP, and CM significantly constituted the highest proportion of total fatty acid content, except for LF. In all four roe oil samples, PUFA levels substantially declined ($p < 0.05$) from M1 to M2 during the ovary maturation process. Immature roe of TA possessed the highest amount of total PUFAs detected, reaching $38.29 \pm 1.04\%$, compared to the other three species. Similar decline trend of PUFA was reported by Phetchthumrongchai et al. (2022) who reported low PUFA content in mature roe of *K. pelamis* compared to immature roe. The majority of PUFA content has been made up of omega-3 fatty acids, including (C18:4) Stearidonic acid, (C20:4) Eicosatetraenoic acid, EPA, Docosapentaenoic acid, and DHA. Corresponding to PUFAS, the percentage of omega-3 fatty acids significantly decreased ($p < 0.05$) with maturation. Nabila et al. (2022) highlighted that omega-3 fatty acids had a significant effect on increasing the gonadal maturation process. Among the n-3 fatty acid group, DHA was the most common fatty acid, followed by EPA. In most marine fish species, DHA shows an increasing trend in roe from immature to mature stages. During early (immature) stages, omega-3 fatty acids are largely stored in somatic tissues and adipose reserves. Within the gonadal development process, these fatty acids are mobilised, converted into lipoproteins, and selectively transported to developing oocytes for yolk formation (Nabila et al., 2022). This trend is evident by the increment of DHA/EPA ratio from immature roe to mature stages.

In TA, KP, and CM, the PUFA contents of the roe samples were arranged in descending order of fatty acid composition as n-3 > n-9 > n-6. However, LF displayed a deviating trend, with n-9 > n-3 > n-6. Similar kinds of trends were previously observed by Nichols et al. (2001) and Georgiou et al. (2023). The DHA/EPA ratio remained constant at around 5.0 in the gonads throughout the three different ovarian phases of TA, KP, and CM. During the development of gonads from the immature stage to the spawning stage, they acquire energy from utilizing lipids. These

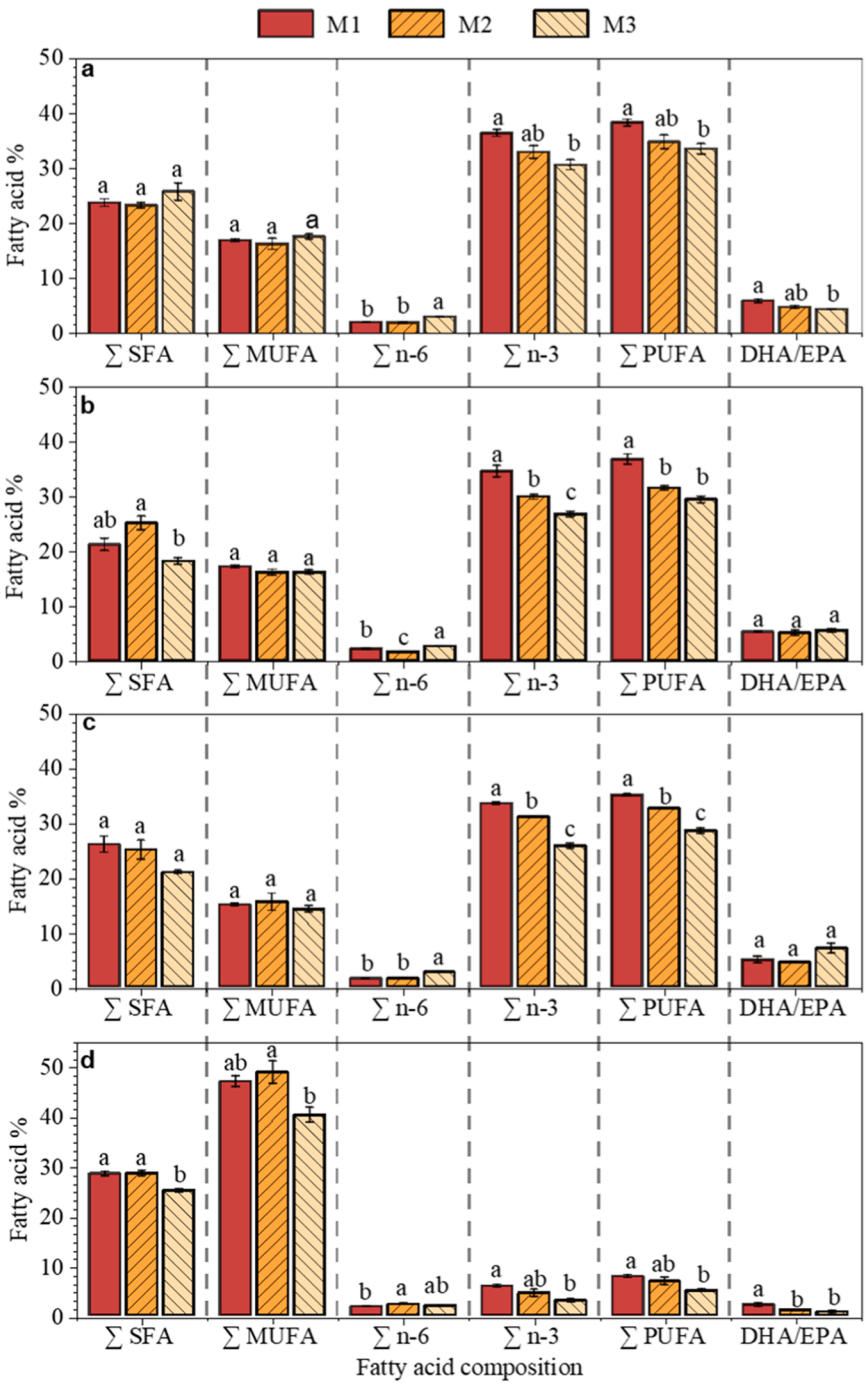

Fig. 4. The variation of the fatty acid composition of roe oil extracted using the solvent extraction method (isopropanol:hexane = 2:1 system) with different maturity stages in four fish species. a: *Thunnus albacares* b: *Katsuwonus pelamis* c: *Canthidermis maculata* d: *Lepidocybium flavobrunneum*; M1: immature M2: maturing M3: matured; ∑SFA: total saturated fatty acids, ∑MUFA: total monounsaturated fatty acids, ∑n-6: total omega-6 fatty acids, ∑n-3: total omega-3 fatty acids, ∑PUFA: total polyunsaturated fatty acids, DHA/EPA: ratio of DHA/EPA; different lowercase letters in each fatty acid category in each roe type differ significantly within each parameter ($p < 0.05$).

lipids can be obtained either exogenously from the maternal diet, mobilized endogenously from adipose fat, synthesized within the ovarian follicle, or transported from other tissues (Sorbera et al., 2001). In the maturation process, the highest total PUFAs were recorded as 36.72 ± 1.63%, 34.97 ± 0.26%, and 7.87 ± 0.55% in the M1 stage of KP, CM, and LF, respectively. Arachidonic acid (C20:4) was detected as the most abundant of n-6 fatty acids, and it was significantly increased with advancing maturity stages. Mature (M3) roe of both tuna species showed the highest ($p < 0.05$) arachidonic acid percentages compared to other species, as 2.33 ± 0.10% in TA and 2.23 ± 0.03% in KP, respectively, while LF showed the lowest ($p < 0.05$). The significant stimulating effect of arachidonic acid in relation to female gonad maturation is highlighted by Sorbera et al. (2001). Additionally, it was found that EPA and other PUFA were crucial for the maturation of marine teleost ovaries. In this study, the roe of TA, KP and CM showed a significant increase of 20:4 n-6 from M1 to M3. But roe oil of LF showed the opposite trend. Therefore, tuna species appear to demonstrate selective catabolism of fatty acids, in this case, selective catabolism of 20:5n-3 relative to 22:6n-3. Overall, selected fish roe can be identified as rich sources of essential fatty acids crucial for promoting human and

animal nutrient requirements. The present findings are consistent with previous results reported by Georgiou et al. (2023) and Tsamesidis et al. (2023) on mullet roe oil composition. Oil extracted from mullet roe has been reported to contain high levels of polyunsaturated fatty acids (25–35%), comparable to the PUFA-rich profiles observed in the current study.

3.6. Estimation of the acid value (AV) and peroxide value (PV) of oils in matured fish roe using the solvent extraction method

Solvent extraction using isopropanol:hexane (2:1) was used to extract roe oil from the matured roe of four species to find the oil quality parameters since M3 roe is ideal for oil extraction.

According to the standard for fish oils recommended by the Codex Alimentarius Commission, the PV and AV of fish oils should be lower than 5 milliequivalents of active oxygen/kg oil and 3 mg KOH/g, respectively (Shanuke et al., 2025). The level of AV is an indication of the oxidation potential of the oil, and PV shows the primary oxidation in the oil. Free fatty acids are highly susceptible to oxidation and fish oil oxidation leads to the occurrence of unacceptable odours and the deterioration of the flavours of the oil (Shahidi and Ambigaipalan, 2018). In the present study, both AV and PV of extracted roe oil were within acceptable limits for human consumption. The lowest acid value was obtained by KP, whereas the highest acid value was observed in TA. There were no significant differences ($p > 0.05$) between the types of roe oil and the PV (Table 2). Hence, the results of AV and PV in the present study suggest that solvent extraction is a promising method to obtain good-quality roe oil.

4. Conclusion

This study shows how nutrient composition, oil recovery, and fatty acid profile of extracted roe oils was significantly influenced by the maturation process. The matured roe of all species showed the highest lipid content and oil recovery, while solvent extraction, specifically using mixed solvents of isopropanol and hexane, was identified as the most efficient roe oil extraction method compared to mechanical pressing, heat hydrolysis, enzymatic hydrolysis, and salt-assisted extraction techniques. GC-MS analysis revealed that the roe oil of *T. albacares*, *K. pelamis*, and *C. maculata* are consisted with high EPA, DHA and other omega-3 fatty acids, whereas roe oil extracted from *L. flavobrunneum* is abundant in oleic acid. The results underscore the potential of underutilized fish roe as a viable source for manufacturing oil rich in essential fatty acids and for developing functional foods with specific fatty acids with well-balanced nutrient composition. Furthermore, this study provides a comprehensive insight into the combined effects of reproductive maturity and various extraction methods on roe oil extraction and composition. Future research is essential to optimize solvent systems, including food-grade solvents like ethanol, enhance extraction efficiency under industrial processing environments; and develop value-added functional foods with improved nutritional and economic benefits. These functional products have the potential to contribute to the sustainable growth of the Sri Lankan fisheries and food processing sector.

CRediT authorship contribution statement

Shanuke D.S: Writing – original draft, Visualization, Software, Methodology, Investigation. **Jayani Chethana Minoli Tissera:** Writing – original draft, Visualization, Validation, Formal analysis. **Coswatte A.C.W.W.M.C.L.K:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **S.C. Jayamanne:** Writing – review & editing, Supervision, Conceptualization.

Table 2
Peroxide Values (PV) and acid values (AV) of extracted roe oil from the four species.

Roe Type	Peroxide Value (meq/kg)	Acid Value (mg KOH/g)
Thunnus albacares	1.01 ± 0.01^{ab}	1.60 ± 0.12^{a}
Katsuwonus pelamis	0.98 ± 0.02^{b}	1.06 ± 0.07^{c}
Canthidermis maculata	1.06 ± 0.05^{a}	1.33 ± 0.09^{b}
Lepidocybium flavobrunneum	1.02 ± 0.01^{ab}	1.21 ± 0.06^{bc}

Values are expressed as mean ± standard deviation (n = 3). Mean values within each column with different superscript lowercase letters differ significantly ($p < 0.05$).

Ethics approval

This research does not require ethical approval.

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Declaration of Competing Interest

A.C.W.W.M.C.L.K.Coswatte reports financial support was provided by Uva Wellassa University. D.S. Sanuke reports on a relationship with Uva Wellassa University that includes investigation and non-financial support. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data Availability

Data will be made available on request.

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