ORIGINAL ARTICLE

Extraction and characterisation of type I collagen from the skin offcuts generated at the commercial fish processing centres

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Abstract Effective use of underutilised fish processing by-products could open avenues for new industries if they are used to extract high-valued bioactive compounds. Therefore, discarded skin offcuts of three main commercial fish species of Sri Lanka were used to extract collagen with acetic acid and the extracted collagen was evaluated for industrial suitability. The yields of acid-soluble collagens from Yellowfin tuna (*Thunnus albacares*), Seer fish (*Scomberomorus commerson*) and Asian sea bass (*Lates calcarifer*) were 61.26%, 58.21% and 59.31%, respectively on a dry-weight basis. Fourier Transform Infra-Red spectra and X-ray Diffraction spectra confirmed that all collagens were in type I and preserved the native triple-helical structure during extraction. The UV absorption spectra confirmed a high collagen purity in all species. These results confirm that the extracted collagen consists of the characteristics required for collagen-based industries such as food, pharmaceutical, and biomedical. The high availability of skin offcuts from the processing industry and the higher collagen yields revealed in this study confirm the possibility of using discarded skin offcuts of the three fish species as a potential source of type I collagen for industrial purposes.

Introduction

Collagen is the most abundant protein, which consists of around 30% of the total body protein, in vertebrates (Li et al. 2013; Singh et al. 2011) and invertebrates (Gobeaux et al. 2008). It is a structural (Chen et al. 2016; Li et al. 2013), extracellular matrix protein (Sun et al. 2017) found in the connective tissues such as skin, bones, tendons and cartilage (Zhang et al. 2016). Although there are 28 genetically distinct collagen types in the collagen family (Ricard-Blum 2011), which differ in their amino acid composition and sequence; molecular structure and function (Li et al. 2013), the type-l collagen, which is the fibril-forming collagen type (Ricard-Blum 2011), is the most abundant in vertebrates and also as the major structural protein in invertebrates (Gobeaux et al. 2008). Type-I collagen has a wide variety of applications in the biomedical; pharmaceutical; food; cosmetic; leather; and film industries (Sionkowska et al. 2015; Zeng et al. 2009).

Mammalian sources are the most common and widely used form of type-I collagen, but its usage has been limited due to the risk of human infections with zoonotic diseases like bovine spongiform encephalopathy or mad cow disease; foot-and-mouth disease and transmissible spongiform encephalopathy (Muralidharan et al. 2013). But fish collagen has not been reported to be linked with such diseases (Li et al. 2013), therefore it has drawn greater attention as a safer alternative to mammalian collagen. Moreover, some

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religious and cultural barriers prevent the use of bovine and porcine origin collagen (Sun et al. 2017). Further, fish collagen has a higher biological value and a higher essential amino acid content than mammalian collagen (Tanaka et al. 2018).

If the fish collagen can be extracted from the fish processing byproducts, which are generally discarded, it could be a great potential for new collagen-based industries because generally, seafood processing recovers only 20–50% as edible portions depending on the processes used and types of products. Simultaneously, 50–80% of remaining off-cuts are discarded as by-products after filleting (Bae et al. 2008), with a global annual average of 20 million Mt (Pal and Suresh 2016). These fish processing by-products generally include damaged muscle, skin, bones, fins and scales (Muralidharan et al. 2013) and they consist of valuable compounds such as collagen, peptides, oils (Lakmini et al., 2022), chitin (Liyanage et al., 2022), vitamins, minerals, enzymes, and pigments which could be used in high-end industries (Pal and Suresh 2016).

In Sri Lanka, most of these fish processing by-products are used for low levels of value addition (Xu et al. 2017) such as fishmeal, fish oil, silage, animal feed, compost and direct fertiliser production while the rest is dumped without getting any use but creating environmental issues. Anyhow, during the last decade, more than 75 medium and large-scale processing plants were established as the export-oriented Sri Lankan fish processing industry was expanding (EDB (Export Development Board (EDB) 2019).

The backbone of the Sri Lankan fish exports industry is Yellowfin tuna (*Thunnus albacares*), and Seer fish (*Scomberomorus commerson*) (Ministry of Fisheries, 2020). Recently, the Asian sea bass (*Lates calcarifer*) or Sri Lankan Barramundi which is coming from aquaculture is also becoming a competitive species contributing to the country's economy. Even though very high quantities of offcuts of these three fish species are generated at fish processing plants, inadequate knowledge hinders its potential usage for extracting bioactive compounds for potential high-end industries. Therefore, the present study was conducted to extract type-I collagen from the discarded skin offcuts of three main commercial fish species, Yellowfin tuna, Seer fish and Asian sea bass to contrast the yields and evaluate the characteristics of extracted collagen to understand the potential of using such discards as a source of type-I collagen.

Material and methods

Raw material

Skin offcuts of Yellowfin tuna (*Thunnus albacares*), Seer fish (*Scomberomorus commerson*) and Asian sea bass (*Lates calcarifer*), which were produced as by-products during the fish filleting process for export were obtained from a fish processing plants located in Ja-Ela, Western Province of Sri Lanka. Samples were brought to the laboratory under frozen conditions. Samples were free of dust and sand as they were already washed at the processing plant, however, they have washed again with distilled water to remove any extraneous matter. They were stored at −20 °C in a freezer until used for the experiment.

Moisture, protein and fat contents

The moisture content of the skins was determined according to the oven drying method (at 105 °C for 24 h). The protein content of the skins and extracted collagen was determined according to the Kjeldahl method. The fat content of the skins was determined according to the method described by Bligh and Dyer (1959).

Pre-treatment of fish skin

From each of the three fish species, a sample of 30 g of the skin was cut separately into 0.3 cm × 0.3 cm pieces (Fig. 1a, c, e) and treated with 0.8 mol/L sodium chloride (NaCl) at a ratio of 1:6 (w/v) for 10 min to remove impurities and pigments. This process was repeated three times and then washed with cold distilled water as described by Muralidharan et al (2013) with modifications as described below. NaCl-treated skins were soaked in cold distilled water at a ratio of 1:50 (w/v) for 5 min with stirring. This washing process was repeated four times. This modification was done to reduce the amount of wastewater. The sodium hydroxide (NaOH) pre-treatment to remove non-collagenous proteins was carried out as described by Sato et al (1986) as follows. The washed samples were soaked in 0.1 mol/L NaOH at a ratio of 1:10 (w/v) for 3 days with gentle stirring. NaOH solution was changed every day. Treated skins were then washed with cold distilled water until neutral pH by soaking in cold distilled water at a ratio of 1:50 (w/v) for 5 min with stirring and repeated four times. The entire process was carried out at 4 °C using cold room conditions.

Extraction of collagen

Acid soluble collagen (ASC) from pre-treated fish skins was extracted following the protocol described by Nagai and Suzuki (2000), with modifications as described below. Samples were treated with 0.5 mol/L acetic acid at a ratio of 1:30 (w/v) for 3 days and then the solution was centrifuged at low centrifugation speed (1,896 × g) for 30 min at 4 °C to cut off the unnecessary production cost. The supernatant was collected, and the residue was re-extracted using the same procedure. The supernatant which was collected from

Fig. 1 Raw skin offcuts of Yel-
lowfin tuna (**a**), Seer fish (**c**) and
Asian sea bass (**e**) on the left
column. The external morphol-
ogy of lyophilised collagen of
Yellowfin tuna (**b**), Seer fish
(**d**), and Asian sea bass (**f**) on
the right column
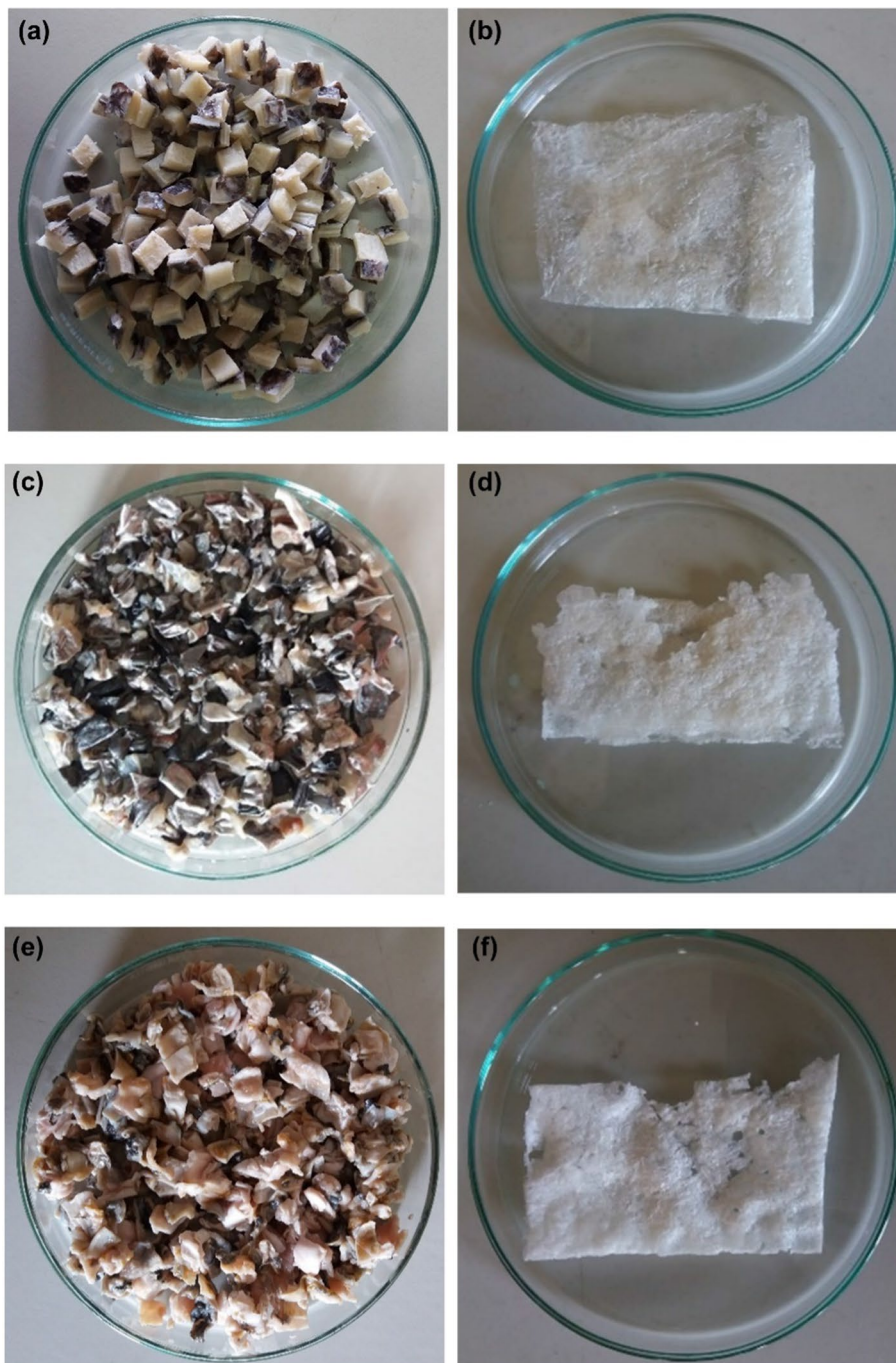
both extractions was salted out by adding NaCl to a final
concentration of 0.9 mol/L. The precipitated collagen was
centrifuged at $1,896 \times g$ for 30 min at 4 °C. The resultant
precipitate was re-dissolved in 0.5 mol/L acetic acid and
dialysed first against 0.1 M acetic acid and for 24 h with a
change of solution at 12 h, and then against distilled water
for 24 h with a change of solution at 12 h, to remove impuri-
ties of chemicals used in the extraction process. The result-
ant solution was then lyophilised and stored at -20 °C until
further experimentation.

Determination of collagen yield

The yield of extracted collagen was calculated according to
the method described by Chen et al. (2016), based on the dry
weight of starting material:

$$\text{Yield (\%)}$$
$$= (\text{Weight of lyophilised collagen/ Weight of initial dry fish skin}) x 100$$

Characterisation of collagen

Scanning electron microscopy (SEM) images

The method described by Zhang et al. (2016) was slightly modified to observe the morphology of collagen. A collagen film of 2 mm was mounted on to aluminium stub using double-sided tape to ensure high electrical conductivity between the specimen and the stub. Then it was sputter-coated with 1 angstrom of gold using a sputter coating machine and viewed in the Carl ZEISS EVO 18 Scanning Electron Microscope using secondary electron mode.

Fourier transform infrared (FTIR) spectra

FTIR spectra of lyophilised collagen samples were obtained to analyse the type of extracted collagen based on the functional groups and the interactions between the bonds. ATR-FTIR spectra of all acid soluble collagen (ASC) were obtained according to the method described by Singh et al. (2011) with slight modifications, using an FTIR spectrophotometer (Bruker Model ALPHA). A collagen film of 5 mm was placed on the crystal cell and the transmittance signals were collected for 40 scans over the range of 4000 to 400 cm^{-1} at a resolution of 2 cm^{-1}. Baseline correction was done using a background spectrum collected from the clean empty cell. Analysis was done at 25 °C. Analysis of spectral data was carried out using the OPUS data collection software program.

X-Ray Diffraction (XRD) spectra

The crystal structures of lyophilised collagen samples were analysed to determine the organisation structure of collagen as described by Alves et al. (2017). The XRD pattern was determined using a CuKα ($\lambda = 1.5405$Å) radiation from Regaku Ultima IV X-ray diffractometer (XRD). A collagen film of 5 cm × 5 cm was placed on general purposes sample holder. Then scan was performed in a range of 2θ = 5 to 30° and at a scanning speed of 2°/min. The crystal phases were identified by using the PDF2 database from International Center for Diffraction Data (ICDD).

UV absorption spectra

UV–Visible spectrophotometric analysis was performed to determine collagen's purity based on the absorption at a specific wavelength. The method described by Zeng et al. (2009) was slightly modified for UV analysis. A sample of 0.2 mg of freeze-dried fish collagen was dissolved in 500 mL of 0.02 M sodium acetate buffer, pH 4.8 containing 2 M urea. The solution was placed into a quartz cell with a path length of 1 mm. The UV spectrum was measured at wavelength 190–400 nm at a scan speed of 2 nm/s with an interval of 1 nm using a Thermo GENESYS 10 UV–Vis Spectrophotometer.

Statistical analysis

Collagen extraction was triplicated for each of the three fish species. For the collagen yield calculations, the mean values were calculated from three determinants and expressed as mean ± standard deviation. The results were statistically interpreted using a one-way analysis of variance (ANOVA) at a 0.05 significance level. Minitab 18 statistical software package was used for the data analysis.

Results and discussion

Moisture, protein and fat content

The moisture content of the fish skin samples and the protein content of both fish skin and extracted collagen are given in Table 1. The percentage moisture contents of the Yellowfin tuna, Seer fish and Asian sea bass were 57.47 ± 0.31, 61.27 ± 0.31 and 61.07 ± 1.94, respectively. The percentage protein contents of the skin of Yellowfin tuna, Seer fish and Asian sea bass were 69.28 ± 0.46, 65.62 ± 0.33 and 65.86 ± 0.11, respectively on dry weight basis while the percentage protein contents of the extracted collagen from the Yellowfin tuna, Seer fish and Asian sea bass were 92.72 ± 0.96, 90.34 ± 1.09 and 92.07 ± 1.39, respectively on dry weight basis. Even though the extracted collagen revealed 90–92% of protein, probably the collagen, the remaining balance could probably be the partially removed lipids and minerals. The slight difference in percentage protein contents of the extracted collagen could probably be

Table 1 Yield of collagen (in dry weight basis), moisture and protein content (in dry weight basis) of the skin and collagen

Fish species	Skin		Collagen		
	Moisture %	Protein %	Protein %	Protein recovery %	Yield %
Yellowfin tuna	57.47 ± 0.31	71.92 ± 0.43	92.72 ± 0.96	78.98 ± 1.07	61.26 ± 0.63
Seer fish	61.27 ± 0.31	67.66 ± 0.23	90.34 ± 1.09	77.73 ± 1.14	58.21 ± 0.27
Asian sea bass	61.07 ± 1.94	68.49 ± 0.14	92.07 ± 1.39	79.73 ± 1.32	59.31 ± 2.87

due to the different lipid and collagen protein contents in the skin offcuts of the three species. The fat contents of Yellowfin tuna, Seer fish and Asian sea bass were $2.7 \pm 0.06\%$, $0.77 \pm 0.05\%$ and $0.81 \pm 0.05\%$ respectively on a wet weight basis. The quality of the extracted collagen is based on the lipid content of the used raw materials since the remaining lipid content directly affects the extracted collagen's odour (Sadowska et al. 2003). If the skin fat is not properly removed during the pre-treatment and remains in the extracted collagen, it develops a rancid fish oil odour (Sadowska et al. 2003). However, the extracted collagen in this study had only a barely detectable fishy odour may be due to the very small amount of fat content in skin offcuts and proper removal of fat during the pre-treatment. Besides, extraction of collagen immediately after receiving the raw materials and storing the extracted collagen in vacuum conditions at -20 °C could have prevented the development of rancid off-odour for upto eight months in this study.

Yield of collagen

Average yields of collagen obtained from skin offcuts of the three fish species were almost up to 60% (Table 1) on a dry weight basis. The remaining wastage could consist of non-collagenous components such as non-collagenous proteins, fat, pigments (Li et al. 2013), and non-extracted enzyme soluble collagen (Bhuimbar et al. 2019). Moreover, no significant differences were obtained among the three species ($P = 0.29$). Different collagen yields have been recorded in previous studies from the skin of different fish species. Anand et al. (2013) reported collagen yields of 8% (Asian Sea bass (*Lates calcarifer*)) and 7.5% (Australasian Snapper (*Pagrus auratus*)) on a dry weight basis, using the acetic acid extraction method. Nagai and Suzuki (2000) reported collagen yields of 51.4% (Japanese seabass (*Lateolabrax japonicus*)), 49.8% (chub mackerel (*Scomber japonicus*)), and 50.1% (bullhead shark (*Heterodontus japonicus*)) on a lyophilised dry weight basis, using acetic acid extraction method. The collagen yield obtained by Li et al (2013) from Spanish mackerel (*Scomeromorous niphonius*) using the acetic acid extraction method was 58.62% on a dry weight basis. A collagen yield of 5.4% on a dry weight basis was obtained from bluefin tuna (*Thunnus orientalis*) by Tanaka et al., (2018) using the combined treatment of acetic acid and pepsin enzyme. Bhuimbar et al (2019) recorded a collagen yield of 25% from black ruff (*Centrolophus niger*) on a wet weight basis. The yield of collagen from the skin may vary due to several factors: fish species; biological conditions of fish such as age; method of extraction i.e. acetic acid extraction, pepsin extraction; parameters of extraction i.e. acid concentration, the ratio of raw materials to acid volume, extraction temperature, time (Pal and Suresh, 2016). Yield data of the present study depict that skin offcuts of these three species are a viable and promising source of collagen to gain economic benefits rather than discarded as waste.

Characterisation of collagen

Morphology

The morphology of the lyophilised collagen was observed under the naked eye and SEM. Collagen film appeared as a white sponge with a porous structure on its surface under the naked eye (Fig. 1b, d, f) for all three fish species. The SEM images of the lyophilised collagen from skin offcuts of three fishes showed the characteristic porous, fibrous and sheet-like morphology which should be preserved for further value-addition of collagen (Fig. 2). Similar morphological results were reported in previous studies using fish as the source of collagen: skin of *Centrolophus niger* (Bhuimbar et al. 2019); skin and bones of *Scomberomorous niphonius* (Li et al. 2013).

FTIR spectra

The FTIR spectra of Yellowfin tuna (Fig. 3a), Seer fish (Fig. 3c) and Asian sea bass (Fig. 3e) exhibited peaks of amide A, amide B, amide I, amide II, and amide III which are characteristics of type I collagen (Alves et al. 2017). These characteristic peaks were similar to the FTIR spectra of other fish species reported in previous studies; tilapia (*Oreochromis niloticus*) (Chen et al. 2016); striped catfish (*Pangasianodon hypophthalmus*) (Singh et al. 2011) and channel catfish (*Ictalurus punctaus*) (Liu et al. 2007). The amide A bands of Yellowfin tuna, Seer fish and Asian sea bass were found at wavenumbers of 3298.81 cm⁻¹, 3281.37 cm⁻¹ and 3379.29 cm⁻¹, respectively. The characteristic infrared absorption of the amide A band is relative to the N–H stretching vibrations (Xu et al. 2017; Li et al. 2013) and is commonly observed in the range between 3,400 and 3,440 cm⁻¹ (Singh et al. 2011). The position shifts to a lower frequency, usually around 3300 cm⁻¹, when the N–H group of a peptide bond is involved in hydrogen bonding probably with a carbonyl group (Singh et al. 2011). The amide B bands of Yellowfin tuna, Seer fish and Asian seabass were found at wavenumbers of 2921.57 cm⁻¹, 2921.90 cm⁻¹ and 2926.01 cm⁻¹, respectively. Amide B band is associated with the asymmetrical stretching of CH_2 groups (Anand et al. 2013; Li et al. 2013) and is commonly observed near 2920 cm⁻¹. The amide I band of Yellowfin tuna, Seer fish and Asian sea bass were found at wavenumbers of 1653.74 cm⁻¹, 1635.30 cm⁻¹ and 1641.42 cm⁻¹, respectively. The amide I band is associated with the stretching vibrations of the carbonyl group ($C=O$ bond) along the polypeptide backbone and is commonly observed in the range from 1,600 to 1,700 cm⁻¹ (Singh et al. 2011). Amide I band
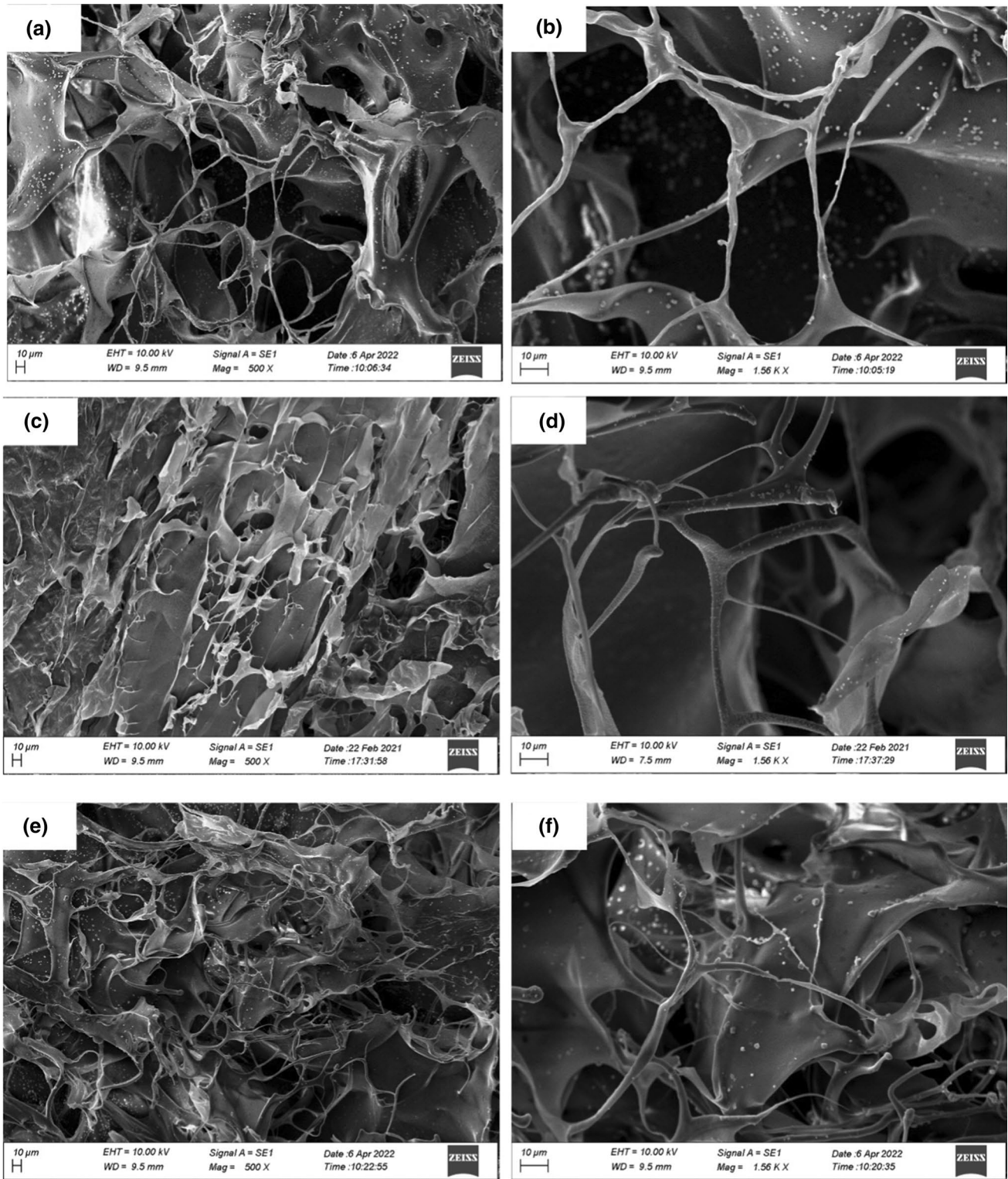
Fig. 2 SEM images of collagen extracted from skin offcuts of Yellowfin tuna (**a**)×500; (**b**)×1,560, Seer fish (**c**):×500; (**d**):×1,560 and Asian sea bass (**e**):×500; (**f**): (×1,560)
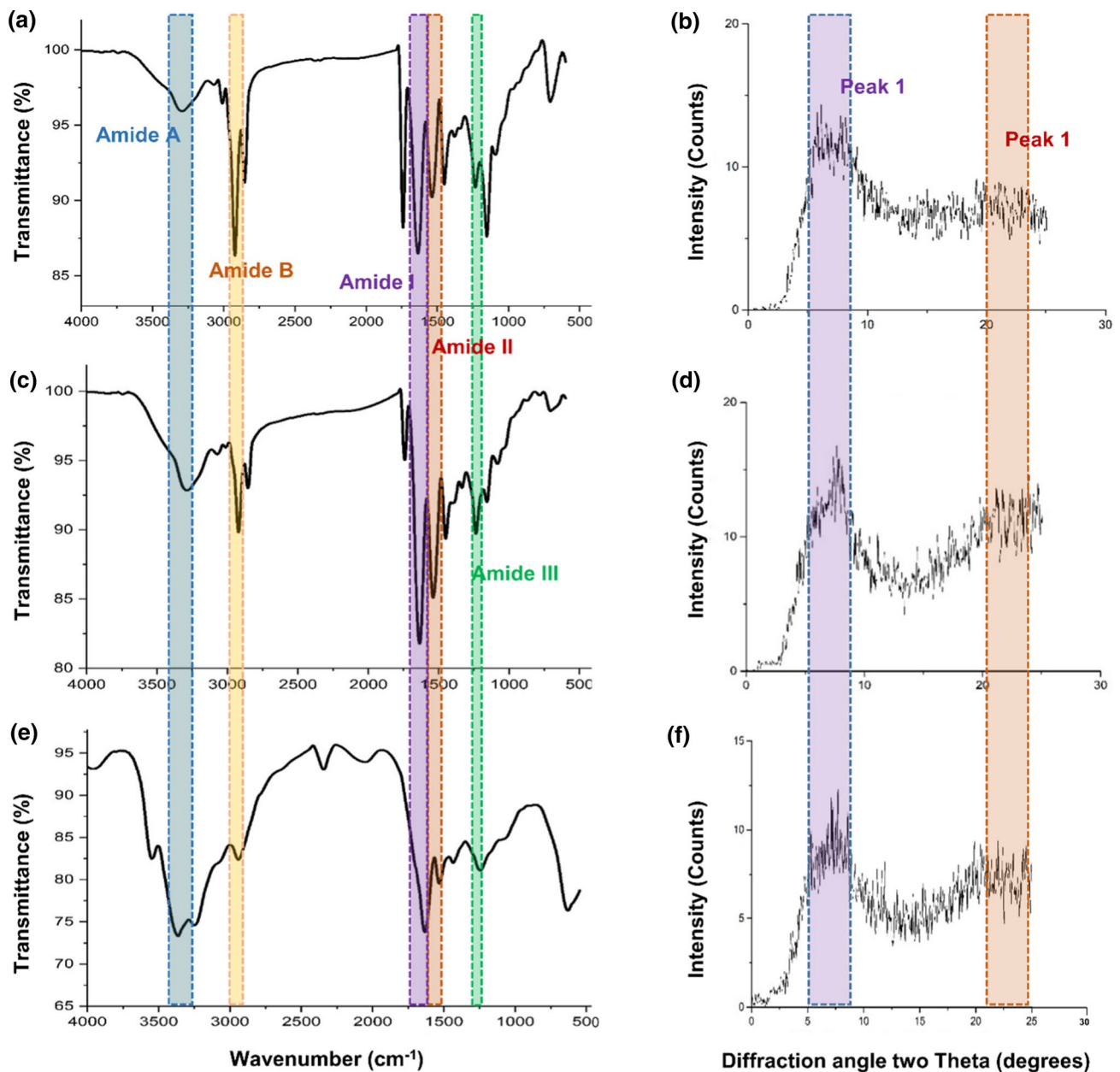
Fig. 3 FTIR spectra of collagen extracted from Yellowfin tuna (**a**), Seer fish (**c**) and Asian sea bass (**e**); XRD spectra of collagen extracted from skin offcuts of Yellowfin tuna (**b**), Seer fish (**d**) and Asian sea bass (**f**)

is responsible for the degree of molecular order found in collagen (Muyonga et al. 2004), and is a sensitive marker of the peptide secondary structure (Singh et al. 2011). The amide II band is commonly observed in the range between 1,550 and 1,600 cm^{-1} (Duan et al. 2009). In this study, amide II bands of Yellowfin tuna, Seer fish and Asian sea bass were found at wavenumbers of 1540.65 cm^{-1}, 1539.74 cm^{-1} and 1539.19 cm^{-1}, respectively. The shifting of amide II band positions to lower frequencies was due to more H bonds indicating a good activity of collagen and triple-helical structure (Riaz et al. 2018). The amide III bands of Yellowfin tuna,

Seer fish and Asian sea bass were found at wavenumbers of 1234.56 cm^{-1}, 1234.83 cm^{-1} and 1247.94 cm^{-1}, respectively. The position of the amide III band around 1240 cm^{-1} is an indication of the existence of a triple-helical structure (Liu et al. 2007). The amide II and amide III bands represent N–H bending vibration coupled with C–N stretching vibration and C–H stretching (Liu et al. 2007; Singh et al. 2011; Riaz et al. 2018).

Additionally, a peak occurred at 1455.37 cm^{-1}, 1451.87 cm^{-1} and 1450.47 cm^{-1}, respectively for Yellowfin tuna, Seer fish and Asian sea bass. An absorption

ratio of approximately 1, between the amide III and the 1454–1450 cm^{-1} peak is an indication of preserving the triple helical structure of collagen molecule during the extraction procedure (Li et al. 2013). In this study, absorption ratio values of 0.98, 1.01 and 0.98 were obtained for Yellowfin tuna, Seer fish and Asian sea bass respectively, indicating that the native triple helical structures were maintained. Native triple helical structure determines the functions of type I collagen, therefore, it is important for further practical applications (Liu et al. 2007). These spectral results confirm that the collagen extracted from the skin offcuts of Yellowfin tuna, Seer fish and Asian sea bass belonging to type I and collagen extraction conditions had no pronounced negative effect on the triple-helical structure. Therefore, the method and conditions provided in this study are suitable to extract collagen from the skin offcuts of these three selected fish species, in non-denatured form.

XRD spectra

As evident in Fig. 3b, d, f, the XRD diagrams of lyophilised collagen extracted from the skin offcuts of Yellowfin tuna, Seer fish and Asian sea bass exhibited two peaks characteristic of collagen (Sun et al. 2017). The Bragg equation: $2d\sin\theta = \lambda$ ($\lambda = 0.15405$), was used to calculate the minimum of the repeated interval (d), where λ is the X-ray wavelength (its value is 1.5405Å), and θ is the Bragg diffraction angle (Wang et al. 2009). The calculated d values are given in Table 2. The first diffraction peak of a collagen XRD spectrum which is relatively sharp is related to the distance between molecular chains of collagen fibres (Alves et al. 2017; Sun et al. 2017). The corresponding diffraction angles (2θ) at the first peak for Yellowfin tuna, Seer fish and Asian sea bass were found at 8.2°, 7.8° and 7.5°, respectively. Accordingly, the calculated distances between molecular chains were 10.77 Å, 11.32 Å and 11.78 Å, for Yellowfin tuna, Seer fish and Asian sea bass, respectively. The distance between molecular chains is important depending on the application of extracted collagen, for example, collagen with a higher distance between molecular chains is more suitable as a drug delivery agent (Chen et al. 2016). The second diffraction peak of a collagen XRD diagram

reflects the diffused scatter caused by many structural layers of collagen fibres (Chen et al. 2016) or in other words the distance between collagen skeletons (Alves et al. 2017; Sun et al. 2017). The corresponding diffraction angles (2θ) at the second peak were found at 20.8°, 24.9° and 22° for Yellowfin tuna, Seer fish and Asian sea bass, respectively. Accordingly, the calculated distance between collagen skeletons was 4.27 Å, 3.57 Å and 4.04 Å, for Yellowfin tuna, Seer fish and Asian sea bass, respectively. These results are similar to the findings of previous studies including fish skin collagen extracted from Tilapia (*Oreochromis niloticus*) by Chen et al. (2016) and Atlantic salmon (*Salmo salar*) by Alves et al. (2017). Calculated distance values were consistent with the diameter of a single left-handed helix chain and a triple-helical collagen molecule, thus confirming the collagen extracted from the skin offcuts of Yellowfin tuna, Seer fish and Asian sea bass were non-denatured with native triple helical conformation (Sun et al. 2017).

UV absorption spectra

Pure type I collagen gives the maximum absorption peak in the wavelength range from 220 to 240 nm. This peak is associated with the $-C=O$, $-COOH$, $-CONH_2$ in polypeptide chains of collagen molecules (Bhuimbar et al. 2019; Zeng et al. 2009). If the extracted collagen is associated with minor amounts of residual amino acids from non-collagenous proteins, a small peak is given at 280 nm corresponding to tyrosine and tryptophan amino acids (Xu et al. 2017; Zeng et al. 2009). Hence, the absence of a peak around 280 nm confirms that pre-treatment has been effective to remove all non-collagenous proteins, and the collagen is pure. In this study, UV absorption spectra were recorded between the 190 nm and 400 nm wavelength range for the three selected fish species (Fig. 4), and the maximum absorption wavelength of the three selected fish species occurred at 217 nm, which was similar to the acid-soluble collagen extracted from several fish species in previous studies including Pacific cod (*Gadus macrocephalus*) skin (Sun et al. 2017); southern catfish (*Silurus meridionalis*) skin (Xu et al. 2017) and Nile tilapia (*Oreochromis niloticus*) skin (Zeng et al.

Table 2 Distance values of XRD peaks of collagen extracted from skin offcuts of Yellowfin tuna, Seer fish and Asian sea bass

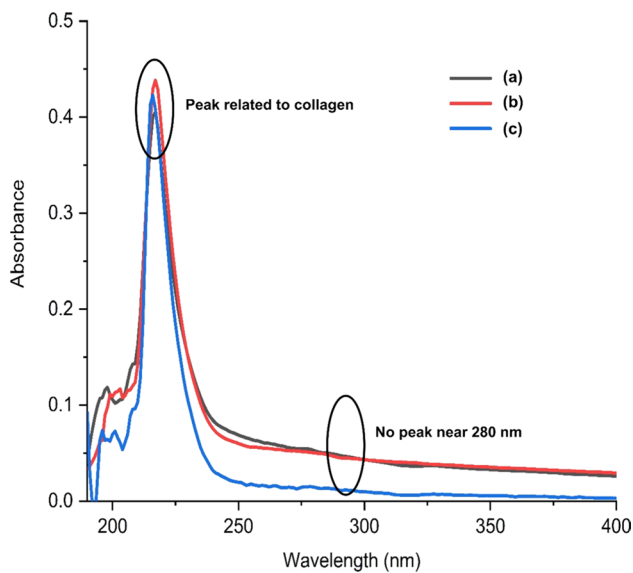| Sample	Peak 1			Peak 2		
	Peak position	Distance between collagen molecules		Peak position	Distance between collagen skeletons	
	2θ	Å	nm	2θ	Å	nm
Yellowfin tuna	8.2	10.77	1.08	20.8	4.27	0.43
Seer fish	7.8	11.32	1.13	24.9	3.57	0.36
Asian sea bass	7.5	11.78	1.18	22.0	4.04	0.40

Fig. 4 UV absorption spectra of collagen extracted from skin offcuts of Yellowfin tuna (**a**), Seer fish (**b**) and Asian sea bass (**c**)

2009). The absence of the peaks at 280 nm of the three species indicates the high purity of collagen.

Conclusion

Collagens were successfully extracted from the skin offcuts of three main commercial fish species in Sri Lanka: Yellowfin tuna (*Thunnus albacares*), Seer fish (*Scomberomorus commerson*) and Asian sea bass (*Lates calcarifer*) using the acetic acid extraction method. Extracted collagen yields were approximately similar from all the three species and were between 55 and 63% on a dry weight basis. All collagens were characterised as type I collagen and maintained their native triple-helical structure which is essential for further value addition. Therefore, the skin offcuts of these three fish species which are discarded in a considerable quantity from fish processing factories can potentially be used to extract collagen for industrial purposes.

Author contribution A.G.D.M.A. did experiments, curated and analysed data, validated data, and wrote the original draft of the manuscript; S.T.G. conceptualised the research idea, curated and analysed data, validated data, engaged in funding acquisition, administered and supervised the project, and reviewed and edited drafts; C.A.N.F. analysed data, engaged in funding acquisition, administered and supervised the project, provided laboratory resources, validated data, and reviewed and edited drafts; M.D.S.T.D.C. conceptualised the research idea, curated and analysed data, engaged in funding acquisition, administered and supervised the project, provided laboratory resources, validated data, and reviewed and edited drafts.

Declarations

Conflicts of interest The authors declare that they have no competing interests.

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