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Characterization of the Thermal Stability of Type I Collagen Extracted From Atlantic Cod (*Gadus morhua*) Skin

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ABSTRACT

Collagen, one of the most abundant structural proteins in vertebrates, is an important compound in biomaterials and biomedicine, with applications in musculoskeletal regeneration and tissue engineering, but also food, packaging, and cosmetics. Its excellent biocompatibility, water-binding capacity, and low cytotoxicity make it highly suitable for these purposes. The chemical structure and functional behavior of extracted collagen vary with its biological source (terrestrial, marine, or recombinant microbial systems) and are strongly influenced by the extraction method, which determines its structural integrity. This work studies the thermal stability of Type I collagen extracted from salted codfish skin at 4°C and subjected to thermal treatment at temperatures from 20°C to 80°C for 72 h. Structural stability and conformational changes were analyzed by UV-Vis, SDS-PAGE, XRD, CD, and FTIR. Results revealed that collagen retained its triple-helical structure up to 20°C, showing no significant deviation from the sample extracted at 4°C. Above 20°C, structural changes are observed, attaining their maximum at 80°C. These results provide insights into the thermal stability of marine collagen and suggest that controlled extraction at room temperature can maintain its structural integrity that can be important toward a sustainable valorization of waste marine biomass.

1 | Introduction

Collagen, comprising at least 28 known types in mammals, represent the most abundant protein family in vertebrates. In humans, 46 genetically distinct collagen α -chains (encoded by 44 genes) have been identified. These chains typically exceed 1000 amino acids and are characterized by a repeating Gly-X-Y triplet motif, where glycine is invariant at every third position to permit tight triple-helical packing. Proline and hydroxyproline frequently occupy the X and Y positions, respectively, providing essential thermal stability, while alanine and other residues contribute to the lateral association of fibrils [1]. It is employed in biodegradable film-forming materials, microencapsulation agents, and numerous biopharmaceutical and biomedical applications [2, 3]. For many of these applications, preserving the molecular integrity

of collagen during extraction is crucial, as its biological functionality is closely linked to its native structural features. In Type I collagen, two identical α 1-chains and one α 2-chain intertwine to form a right-handed triple-helical structure (THS), which is fundamental to the mechanical strength of collagen in tissues [4]. The amino acid composition, combined with the precise assembly of the polypeptide chains, post-translational modifications, and additional reinforcement by interchain hydrogen bonding and electrostatic interactions, ultimately leads to the formation of a collagen molecule with approximately 300 nm in length and 1.4 nm in diameter [5]. Collagen's molecular conformation is reported not to be always a helix [6]. Depending on the amino acid composition, chain assembly, and post-translational modifications, collagen molecules can also form β -chains (dimers) and γ -chains (trimers). Traditionally, collagen has been sourced

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from terrestrial animals such as bovine and porcine. However, these sources are becoming less favored due to limited availability, ethical and religious concerns, and risks associated with zoonotic diseases, underscoring the need for more sustainable alternatives [7]. Recent advances have expanded the use of marine collagen across various sectors. Fish skin, a by-product of fish fillet processing, is a valuable source of Type I collagen and offers significant potential for valorization through diverse extraction methods and solvents [8]. Its distinctive properties, such as biocompatibility and low antigenicity, make collagen and its derivatives particularly attractive for a wide range of applications including medical, cosmetic, nutraceutical, food, and pharmaceutical sectors [9]. Notably, codfish skin-derived collagen hydrolysates have been investigated for their role in enhancing anthocyanin stability [10], a significant source of γ -chain components [2, 7].

Several parameters, such as fish species, habitat temperature, and intrinsic thermal stability, significantly influence the characteristics and biochemical properties of this collagen [11]. Marine collagen polypeptide chains exhibit considerable variation in amino acid composition, particularly in hydroxyproline and proline content, which differ across species, and environmental temperature. These variations directly impact collagen's thermal stability [12]. Additionally, the extraction process itself can modify the native molecular structure and alter key functional properties, including water absorption capacity, solubility, viscosity, and molecular functionality [11, 13, 14].

The extraction of collagen from fish by-products typically involves several key steps, namely biomass pretreatment, collagen extraction, and recovery [15, 16]. Due to the stabilization of the THS by extensive hydrogen bonding, collagen is typically insoluble in water. To enhance the extraction efficiency and improve solubility, various approaches, such as the use of chemical solvents, enzymatic treatments, or advanced extraction techniques, are often used [13, 17, 18]. Among these, acetic acid and (deep) eutectic solvents (DESSs) have been reported on the extraction of marine collagen, with the goal of preserving the molecular integrity of collagen and ensuring that the extracted collagen retains its desired biomedical functionality [11, 19, 20]. As previously mentioned, various extraction parameters, such as acid concentration, extraction time, solid-to-liquid ratio, and temperature, have a significant impact on collagen yield, purity, and its biological and molecular functional properties [8, 14]. Among these factors, temperature plays a critical role, as excessive heat can degrade collagen and decrease its biofunctionality [11]. High temperatures disrupt intermolecular interactions, particularly hydrogen bonding between the amide hydrogen and carbonyl oxygen in the α -chains, essential interactions for α -helix formation and the maintenance of the THS of collagen [21, 22]. Although marine collagen is typically extracted at low temperatures (around 4°C) to preserve its native structure, this practice increases operational costs and extends processing times. Prolonged exposure to higher temperatures can also degrade the collagen's THS, especially in collagen from cold-water species like codfish, which contain lower levels of hydroxyproline and exhibit poor thermal stability compared to collagen from terrestrial sources [3, 8, 11, 15, 23, 24]. It is recurrently reported the sensitivity of marine collagen to temperatures above 15°C, which has been dictating the extraction processes temperature, typically maintained around the 4°C [11, 25, 26]. Therefore, controlling the temperature throughout

the extraction process is critical to maintain the structural integrity of the molecule (preventing the dissociation of the intermolecular interactions within the α -chains and THS), biocompatibility, and bioactivity [27].

While conventional protocols for cold-water fish collagen extraction mandate low temperatures (typically 4°C) to prevent thermal denaturation, the necessity of these energy-intensive conditions for preserved biomass remains an unresolved question. Building upon the work of Bisht et al. [11], who reported collagen extraction from salted codfish skin at 4°C, this study investigates whether such stringent cooling is required to maintain structural integrity. In this study, we employed multiple analytical techniques to investigate the thermal stability of Type I collagen extracted from salted codfish skin at 4°C, using rat tail collagen as a reference. The samples were subjected to thermal treatments at temperatures from 20°C to 80°C for 72h. The aim was to characterize temperature-dependent structural changes using complementary methods and assess the feasibility of extracting collagen from salted codfish skin at ambient temperature while preserving its native structure. The extraction strategy was deliberately not expanded to multiple extraction temperatures. Instead, the study was designed to evaluate temperature as an isolated variable, to evaluate the structural response of the obtained collagen (by conventional method) under controlled heating conditions, and to maintain a constant extraction baseline, rather than a process variable, in order to decouple extraction-driven structural alterations from thermal effects occurring during the extraction process.

Fourier-transform infrared spectroscopy (FTIR) is a well-established and versatile technique for the analysis of proteins and peptides, being particularly effective in probing the chemical structure of collagen. It enables the differentiation of major collagen types, including Type I collagen, by examining key spectral regions associated with characteristic amide bands [28]. The technique reveals distinct spectral patterns arising from the THS of collagen composed of α -chains arranged in the Gly-X-Y repeating motif [5, 29]. Other techniques such as ultraviolet-visible (UV-Vis) spectroscopy, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), X-ray diffraction (XRD), and circular dichroism (CD) are used as complementary analytical tools for collagen characterization, confirming collagen type, molecular integrity, secondary structure, and crystallinity [11].

2 | Materials and Methods

2.1 | Material, Methods, and the Extraction Process

Atlantic cod (*Gadus morhua*) skins were generously supplied by Pascoal & Filhos, S.A. (Aveiro, Portugal). The skins were collected cold from the factory and carried on ice, transported to the laboratory at the University of Aveiro, and stored at -20°C for further use. Commercial rat tail collagen was sourced from Gibco (REF. A10483-01), while cholinium chloride (>97%), sodium chloride (99.5%), and sodium hydroxide (97%) were purchased from Acros Organics, and acetic acid (99%) and butyl alcohol (99%) were obtained from Fisher Scientific. Eppendorf ThermoMixer F1.5 was used for thermal treatment. The codfish skins were manually cleaned and rinsed with cold distilled water. The skins were then

cut into small pieces, for further use. All steps from pretreatment, extraction, and recovery of collagen were adopted from Bisht et al. [11] and slightly adapted to suit the experimental aims of this work. The DES was prepared by dissolving in water-specific HBA and HBD chemicals at a molar ratio of 1:2. The pH of the DES was measured at room temperature using a Mettler Toledo S47 SevenMulti dual meter for pH and conductivity, with an accuracy of ± 0.01 displaying a value of 2.60 (± 0.02). Rat tail collagen (Gibco [REF. A10483-01]) was selected as the collagen of reference (standard) in this study. Salted codfish skins used in this study were obtained from a single batch to ensure raw material consistency. Collagen extraction method was performed once. The resulting collagen was analyzed in triplicate for each condition (20°C–80°C) to ensure analytical reproducibility.

2.2 | Collagen Identification and Structural Verification

2.2.1 | UV-Vis Spectrum

UV-Vis spectrum analysis for detecting the presence of protein in our samples was adopted from literature [11, 30]. The samples were dissolved in acetic acid at 0.5% at a concentration of 0.5 mg mL^{-1} , and the resulting solutions were subjected to UV-Vis spectrophotometry between 200 and 400 nm, with the baseline calibration performed using acetic acid at 0.5%.

2.2.2 | SDS-PAGE

SDS-PAGE analyses were carried following a method adapted from Bisht et al. [11]. They were conducted using polyacrylamide gels (stacking: 4%, resolving: 20%) and a running buffer (pH 8.3) composed of 250 mM Tris-HCl, 1.92 M glycine, and 1% SDS. The collagen samples were stained following the standard protocol with Coomassie Brilliant Blue [11].

2.2.3 | XRD

XRD analysis was performed at room temperature using an Empyrean (Malvern Panalytical) diffractometer equipped with $\text{CuK}\alpha$ radiation ($\lambda = 1.54018 \text{ \AA}$, 45 kV, 40 mA). Data were collected over a 2θ range of 3° – 30° at a scan rate of $0.0263^\circ \text{ s}^{-1}$.

2.2.4 | CD

CD spectra of the extracts were obtained in the 190–280 nm range using a 0.1 cm path-length cuvette. Lyophilized collagen was dissolved at 0.5 mg mL^{-1} in 0.5% acetic acid and incubated at 4°C for 6 h. The solvent spectrum was subtracted to correct the sample baseline. Each spectrum represents the average of three scans, recorded with a bandwidth of 1.0 nm and a response time of 1 s.

2.2.5 | Fourier-Transform Infrared Spectroscopy (ATR-FTIR)

For disk preparation, 3 mg of freeze-dried collagen samples was pressed into pellets using a hydraulic press at 10 t for 20 s without KBr for analysis. Extracted collagen molecules were analyzed using an ATR-FTIR spectrometer (JASCO FT/IR-4X1 type A). The spectra were recorded over a range of 4000 – 400 cm^{-1} by accumulating 128 scans at a resolution of 4 cm^{-1} . After measuring all FTIR

spectra for each sample, background subtraction was performed, and the average spectra were calculated.

2.3 | Thermal Treatment

To investigate the thermal stability of collagen samples, these were exposed to four temperatures (20°C, 30°C, 40°C, and 80°C) for 72 h. Importantly, the thermal degradation was performed without the use of chemical agents such as strong acids or bases, ensuring that the native structure of collagen remained unaltered by external chemicals. This method allowed for a focused evaluation of temperature's direct impact on collagen's intra-intermolecular hydrogen bonding, as revealed through changes in the amide bands in the FTIR spectra as well as other analysis.

3 | Results and Discussion

Collagen was extracted from salted Atlantic cod (*Gadus morhua*) skin using an aqueous solution of a DES at 4°C, with commercial rat tail tendon collagen serving as the reference. After extraction, 10 mL of each collagen solution (commercial and codfish-derived) was freeze-dried, and 3 mg of dried collagen was dissolved in 1 mL of 0.05 M acetic acid in 2 mL microtubes, followed by gentle stirring at 50 rpm overnight at 4°C until fully solubilized. The resulting solutions (commercial and codfish-derived) were thermally processed at 20°C, 30°C, 40°C, and 80°C, for 72 h using a ThermoMixer, while reference samples were maintained at 4°C as described in Table 1. After heating, all samples were stored at -80°C overnight, freeze-dried, and subsequently prepared for analysis according to method-specific requirements. This design enabled controlled comparison of temperature-dependent structural changes across collagen sources and treatments.

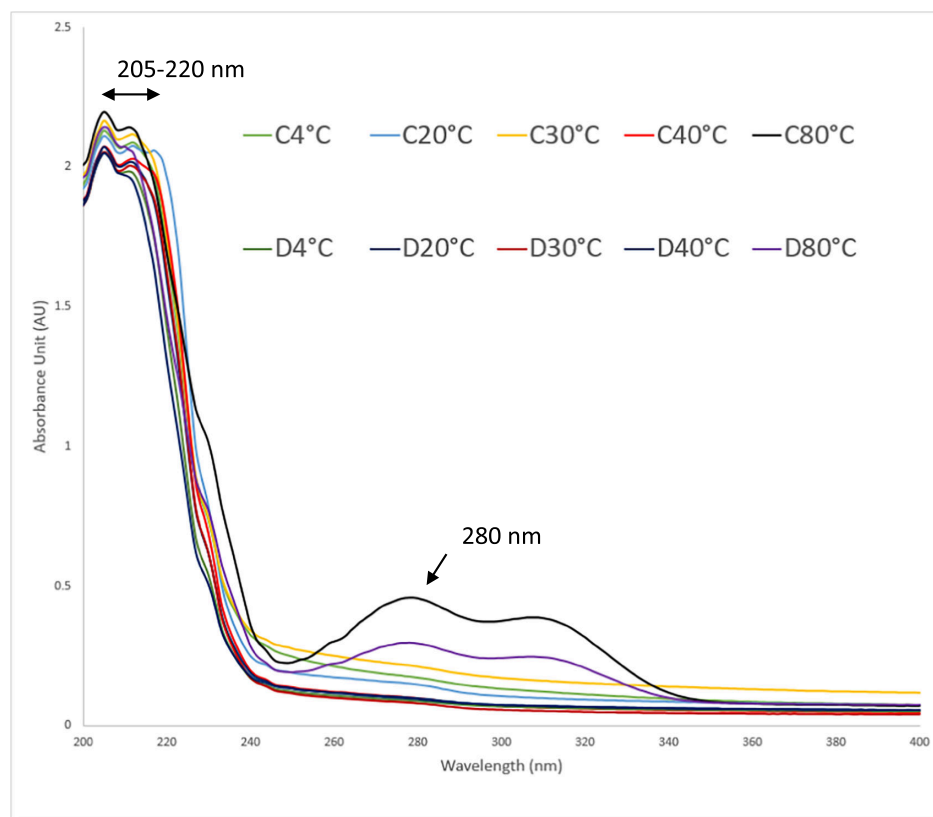
3.1 | UV-Vis Spectra of the Collagen Samples

The presence of collagen in the samples studied is expected to be detected in the range of 200–230 nm [11]. The UV-Vis peak near 220 nm is characteristic of C=O, $-\text{COOH}$, and $\text{CO}-\text{NH}_2$ groups in collagen polypeptide chains and indicative of the presence of the THS [19]. However, UV-Vis spectroscopy was employed as a preliminary method to assess protein presence and extract purity rather than to specifically identify collagen. The absorption in the 200–230 nm region corresponds to peptide bonds and is indicative of proteinaceous material, while absorbance near 280 nm was used to evaluate the presence of noncollagenous proteins. Therefore, this analysis was used to confirm protein presence and assess relative purity of the extracts.

The commercial and extracted cod fish collagen samples showed absorption bands within 205–220 nm. Except for the samples treated at 80°C, no band was observed at 280 nm indicating the absence of noncollagenous proteins on the samples (Figure 1). A distinct band near 205 nm, indicative of the characteristic structure of collagen, was observed in all samples (Figure 1). The band around 280 nm corresponds to the presence of aromatic side chains, primarily tryptophan, phenylalanine, and tyrosine. Since Type I collagen lacks tryptophan residues, the enhanced absorbance at 280 nm following prolonged thermal treatment

TABLE 1 | Collagen samples, preparation workflow, and thermal treatment conditions.

Collagen source	Sample name	Preparation conditions	Thermal treatment	Post-treatment handling
Commercial rat tail Type I collagen	C4°C	3 mg freeze-dried collagen dissolved in 1 mL 0.05 M acetic acid; stirred overnight at 4°C	4°C (reference)	All samples stored at −80°C overnight, freeze-dried, and prepared for UV-Vis, SDS-PAGE, XRD, CD, and FTIR-ATR analysis [11]
	C20°C	Same as C 4°C	20°C	
	C30°C	Same as C 4°C	30°C	
	C40°C	Same as C 4°C	40°C	
	C80°C	Same as C 4°C	80°C	
Codfish (<i>Gadus morhua</i>) skin collagen extracted using aqueous DES at 4°C	D4°C	3 mg freeze-dried collagen dissolved in 1 mL 0.05 M acetic acid; stirred overnight at 4°C	4°C (reference)	
	D20°C	Same as D 4°C	20°C	
	D30°C	Same as D 4°C	30°C	
	D40°C	Same as D 4°C	40°C	
	D80°C	Same as D 4°C	80°C	

**FIGURE 1** | Protein detection in the range of 200–400 nm, while the presence of collagen was detected near 205 nm. Samples C80°C and D80°C showing a peak near 280 nm. C: commercial collagen (rat tail Type I collagen). D: fish skin collagen extracted by DES.

can be attributed to peptide chain cleavage and the consequent exposure of phenylalanine and tyrosine residues [11].

3.2 | SDS-PAGE

The SDS-PAGE results for all collagen samples are presented in Figure 2. The α 1- and α 2-chains with molecular weights near

115 kDa and β - and γ -chains, representing dimers and trimers of the α -chains, with molecular weights exceeding 230 kDa, were detected. The results are in good agreement with commercial collagen (C) data; however, as expected, codfish skin collagen showed a higher proportion of γ -chains compared to commercial collagen [31]. The relative proportion of γ -components in marine collagen can vary depending on species and biomass condition, often exhibiting multiple γ -bands (e.g., γ 1, γ 2, and

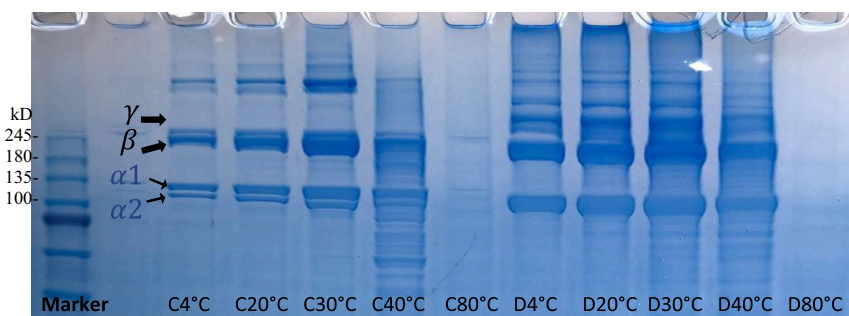


FIGURE 2 | SDS-PAGE analysis of collagen samples. Marker: molecular weight standard. C: commercial collagen (rat tail Type I collagen). D: fish skin collagen extracted by DES (D).

γ 3) associated with intermolecular cross-linking [13, 31]. In this study, the use of salted and semidried codfish skin may contribute to a relatively higher proportion of γ -components compared to commercial collagen, likely due to biomass pretreatment and/or processing conditions.

The α 1- and α 2-chains, with molecular weights of approximately 115 kDa and the β - and γ -chains, corresponding to dimers and trimers of the α -chains with molecular weights above 230 kDa, were identified for all samples except samples treated at 80°C (Figure 2); however, the electrophoretic patterns of thermally treated samples at 20°C were similar to samples at 4°C for both commercial and cod fish skin collagen samples, indicating that treatment at 20°C did not induce structural degradation. Commercial collagen exhibited a distinct decrease in band intensity after treatment at 40°C, with bands becoming nearly undetectable at 80°C. Codfish collagen showed earlier structural destabilization, with chain cleavage initiating at 30°C as evidenced by the appearance of low-molecular-weight bands indicative of partial thermal degradation. Band intensities decreased further at 40°C and disappeared completely at 80°C (Figure 2).

3.3 | XRD

The disks of the lyophilized collagen samples prepared for FTIR were used for XRD studies. All collagen samples, except for samples treated at 80°C showed a characteristic peak at diffraction angles (2θ) ranging from 7.5° to 9° for commercial and cod fish skin collagen, corresponding to the THS, and a broad diffraction peak around $\sim 20^\circ$, which is attributed to amorphous scattering (Table 2 and Figure 3) [11].

The XRD patterns of commercial collagen displayed a diffraction peak at $2\theta \approx 7.5^\circ$, characteristic of the triple-helical collagen structure and indicative of its ordered crystalline regions. For codfish collagen, a similar diffraction feature was observed at $2\theta \approx 7.8^\circ$, suggesting a slightly more compact molecular packing, likely influenced by the initial biomass condition (salted codfish skin) and the extraction process. According to Bragg's law, the corresponding d-spacings were calculated for commercial and codfish collagen samples (Table 2). Notably, collagen samples thermally treated at 20°C exhibited diffraction peaks like those at 4°C, suggesting that the native structural order of both commercial and codfish collagen samples was preserved under these conditions. In contrast, the diffraction peak of both commercial and codfish collagen treated at 80°C for 72 h disappeared, reflecting thermal

denaturation and loss molecular packaging (Figure 3 and Table 2). The commercial collagen samples treated at 30°C retained a diffraction peak near $2\theta \approx 7.7^\circ$, although the sample treated at 40°C is in much lower intensity and broader band shape compared to the profile of the sample treated at 4°C and 20°C. In contrast, codfish collagen treated at 30°C showed a more attenuated and broadened peak in this region, reflecting greater disruption of molecular order (Figure S3).

3.4 | CD

CD spectroscopy is commonly employed to evaluate protein secondary structures. Figure 4a,b presents the CD spectra of the studied samples. Both positive and negative characteristic bands were revealed in commercial Type I collagen. In contrast, the codfish collagen sample lacked the positive band around 222 nm, which is indicative of a THS. The pronounced negative minimum near 196 nm corresponds to a random coil conformation [11].

CD analysis revealed that both commercial and codfish collagen maintained their secondary structure at 20°C same as at 4°C, as indicated by the ellipticity band at 222 nm (θ_{222}), ellipticity bands at 199 nm (θ_{199}), and consistent $\theta_{222}/\theta_{199}$ ratios (Figure 4 and Table 2). The $\theta_{222}/\theta_{199}$ ratios were calculated based on Equation (1) [32, 33].

$$[\theta] = \frac{(\text{MRW} \times \theta_{\text{obs}} \times 100)}{c \times l} \quad (1)$$

where θ is the mean residue ellipticity, in $\text{deg cm}^2 \text{dmol}^{-1}$, MRW is the mean residue weight of the protein (Da/residue), θ_{obs} is the observed ellipticity from the CD spectropolarimeter (millidegree [mdeg]), c is the protein concentration, in mg mL^{-1} , and l is the light path length, in cm.

For commercial collagen, θ_{222} decreased slightly from +24.49 at 4°C to +20.63 at 20°C, with the $\theta_{222}/\theta_{199}$ ratio remaining near 0.12–0.13, indicating minimal structural changes over 72 h. Codfish collagen exhibited similar stability, with θ_{199} values of –47.09 and –45.23 with the $\theta_{222}/\theta_{199}$ ratio remaining near 0.092–0.078 at 4°C and 20°C, respectively. Thermal exposure at 30°C and above led to progressive loss of the secondary structure for both collagen samples. The θ_{222} decreased to +1.73 and θ_{199} decreased to –72.48 at 30°C and further to –1.87 and –30.11 at 80°C for commercial collagen, accompanied by a decline in $\theta_{222}/\theta_{199}$ ratios (Table 2). θ_{222} and θ_{199} significant changes at 30°C and 40°C and

TABLE 2 | Comparative analysis of spectroscopic, electrophoretic, and crystallographic assessment of thermal degradation of Type I collagen from codfish skin.

Sample	Temperature, °C	UV-Vis 200–250 nm (AUC)	SDS–PAGE ^a $\alpha/\beta/\gamma$ integrity	Amide III/ 1450 cm ^{−1} absorption ratio	CD mean residue ellipticity, $\theta\theta_{222}/\theta_{99}$ ratios	XRD 2θ , °					Collagen fibrillar order	
						Index	Area	FWHM	Center	Height		d-spacing, Å
Commercial	4	85.07	Intact	1.04	0.13	1	1.091	1.48	7.52	0.63	11.77	Retained
collagen	20	85.15	Intact	1.1	0.12	2	4.724	8.11	21.47	0.54	4.13	Retained
						1	0.969	1.56	7.60	0.54	11.62	
	30	85.23	Intact	1.1	0.03	2	3.372	8.76	20.84	0.39	4.25	Retained
						1	1.011	1.53	7.72	0.59	11.43	
Codfish collagen	40	87.6	Faded	1.00	0.02	2	2.421	8.24	19.89	0.29	4.46	Mainly distorted
						1	0.705	—	7.70	0.25	11.45	
	80	89.55	Disappeared	1.04	—	2	3.453	6.18	19.93	0.51	4.45	Completely distorted
						ND	—	—	—	—	—	
Codfish collagen	4	86.8	Intact	1.01	0.09	1	4.053	6.05	20.76	0.56	4.27	Retained
						1	1.656	2.38	7.80	0.58	11.31	
	20	86.95	Intact	1.02	0.07	2	4.987	7.35	20.05	0.67	4.42	Retained
						1	1.633	2.11	7.96	0.62	11.10	
Codfish collagen	30	87.94	Intact with low molecular fragments	0.99	0.11 ^b	2	3.639	6.40	21.78	0.53	4.07	Partially distorted
						1	1.021	2.38	8.70	0.30	10.15	
	40	88.32	Faded with low molecular fragments	1.01	0.10 ^b	2	4.787	5.68	19.42	0.70	4.57	Highly distorted
						1	0.899	—	9.06	0.28	9.75	
Codfish collagen	80	88.87	Disappeared	0.99	—	2	3.650	5.55	19.58	0.59	4.53	Completely distorted
						ND	—	—	—	—	—	

Note: The Full Width at Half Maximum (FWHM) values obtained for samples at 40 °C (both commercial and codfish collagen) were not considered reliable, as they result from fitting of very weak and poorly defined diffraction features, therefore excluded from the report.
Abbreviation: ND, not detected.
^aSDS–PAGE analysis is presented solely to assess molecular weight distribution and collagen hydrolysis level, as its denaturing conditions prevents evaluation of triple-helix structural integrity.
^bThe absolute ellipticity value is much smaller than collagen samples at 4 °C, and this ratio does not reflect the helical structure integrity.

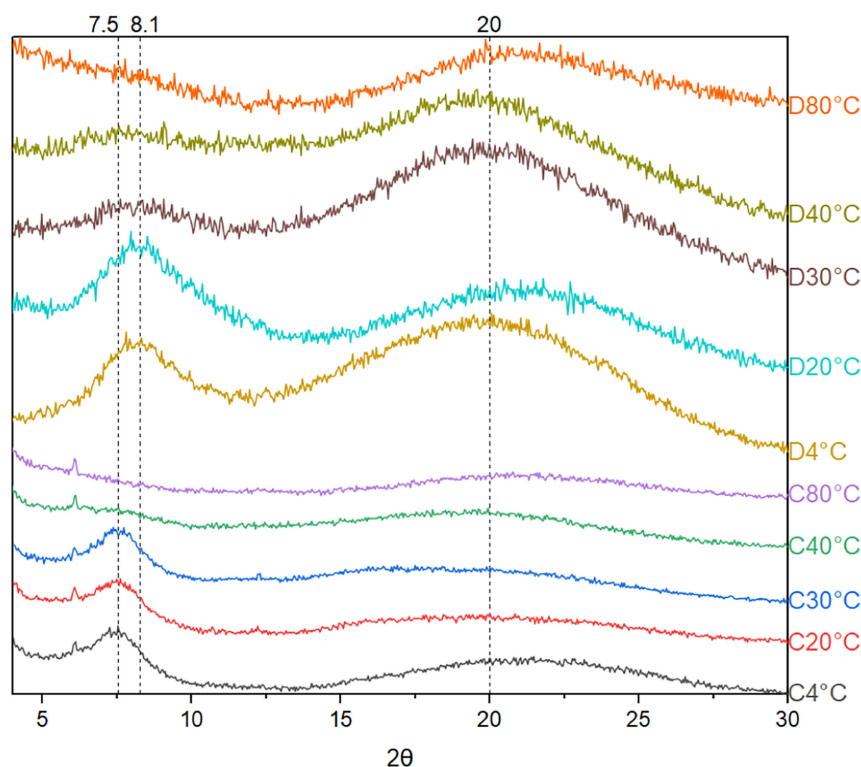


FIGURE 3 | X-ray diffraction of the collagen samples, 2θ ranging from 3° to 30° . C: commercial collagen (rat tail Type I collagen). D: collagen extracted from cod fish skin by DES.

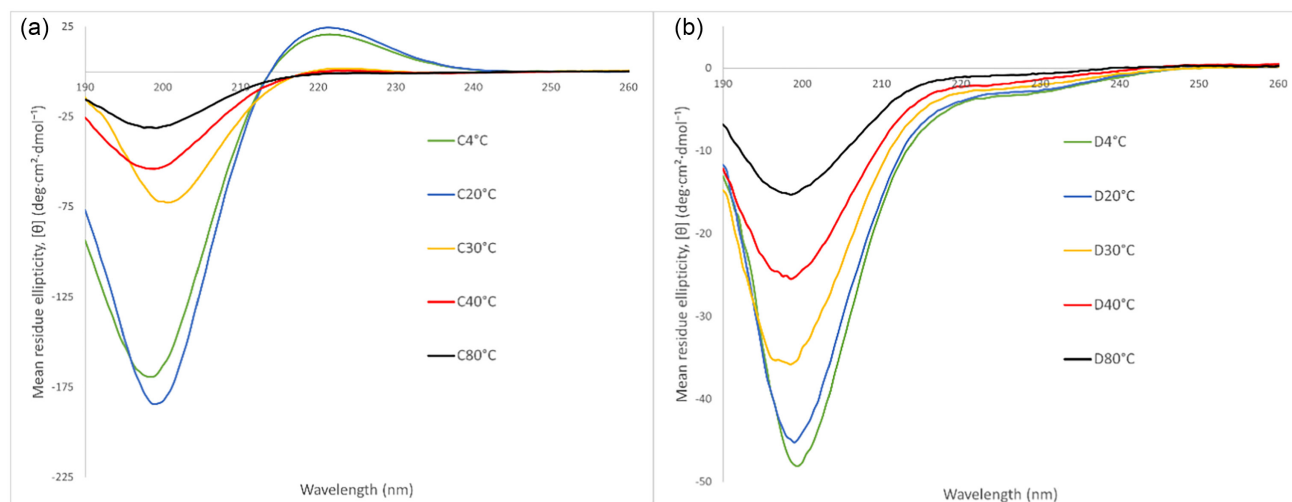


FIGURE 4 | CD spectra of the collagen samples, ranging from 280 to 190 nm. (a) Commercial collagen (rat tail Type I collagen [C]). (b) Collagen extracted from cod fish skin by DES (D).

further to -1.41 and -30.28 at 80°C for codfish collagen represent the secondary structure alteration. These results, from CD and further FTIR data, confirm that collagen structural integrity is maintained under mild conditions ($<30^\circ\text{C}$) yet is thermally sensitive, with temperatures of 30°C and above inducing denaturation and peptide chain exposure. For collagen samples with higher $\theta_{222}/\theta_{199}$ ratio ($\text{C}80^\circ\text{C}$, $\text{D}40^\circ\text{C}$, and $\text{D}80^\circ\text{C}$), the absolute ellipticity values are much smaller than initial (samples at 4°C) and the ratio does not reflect the magnitude of the helical structure. The absolute θ_{222} and θ_{199} must be reasonably large to calculate the $\theta_{220}/\theta_{198}$ ratios, and when it drops more than 50% of the initial θ_+ , it indicates secondary structure changes (Figure 4 and Table 2)

[32, 34]. The lower Rpn values observed for codfish skin collagen compared with commercial rat tail collagen are attributed to partial loss of the THS and are likely associated with the reduced proline/hydroxyproline content of marine collagen relative to mammalian collagen [35].

3.5 | FTIR-ATR Spectroscopy

FTIR is particularly relevant for studying collagen's chemical structure. This technique is essential as it effectively analyzes the main amide bands in Type I collagen, providing critical insights

into its chemical properties and structure, which may be relevant for diverse applications [36]. The Gly-X-Y motif configuration of intertwined α -chains forming a THS produces distinct patterns in FTIR analysis [5, 29]. The FTIR spectra of all collagen samples analyzed are presented in Figure 5, and it shows the characteristic peaks corresponding to Amide I, II, and III and Amide A and B. The major peaks observed in the spectra of collagen extracted from codfish skins were consistent with commercial collagen and collagen recovered from marine sources reported in previous studies [11, 23, 37–39]. The secondary structure and the Amide I, II, and III bands will be discussed below based on data from previous studies [6, 29, 40–44].

3.5.1 | Characteristic Amide Bands

For Amide A, a free NH-stretching vibration typically occurs in the $3400\text{--}3440\text{ cm}^{-1}$ range, while when the NH group of a peptide is engaged in hydrogen bond, this position shifts to lower frequencies, generally around 3300 cm^{-1} . Moreover, the stronger the hydrogen bond established, the lower the frequency of this band would be [44]. In collagen, the hydrogen bonds are weaker compared to other proteins. Consequently, the Amide A band is typically observed at a higher frequency near the 3330 cm^{-1} . However, its position in triple-helical polypeptides is reported to occur at higher frequency than in other proteins, likely due to supercoiling of the triple helix. Moreover, increased supercoiling may lead to less favorable bond angles and longer hydrogen bonds, which could explain the high frequency observed in collagen [44, 45]. It has also been suggested that longer hydrogen bonds affect the band intensity more significantly than they cause the shift in the band position [46]. In our data, the Amide A peak for all collagen samples appeared near 3270 cm^{-1} , indicating that the NH

groups were involved in hydrogen bonding (Figure 5). The Amide B band, associated with NH stretching vibrations, was observed near 3080 cm^{-1} for all collagen samples, indicating hydrogen bonding involving NH groups (Figure 5). In collagen's triple helix, weak hydrogen bonding and the influence of N–H bending overtone slightly shift the Amide B band to lower frequencies compared to α -helices or β -sheets [29]. Despite the stable N–H bond length, the dense packing of the helix restricts vibrational coupling, often positioning the Amide B band near 3070 cm^{-1} [47–49]. Furthermore, the hydration level of collagen also affects the hydrogen bond strength, often resulting in a downward shift of this band [50].

The Amide I band, primarily arising from C=O stretching in the peptide bond, is highly sensitive to triple helical secondary structure and serves as a key indicator of collagen conformation, normally appearing around 1650 cm^{-1} in Type I collagen [42, 51]. In cold-water marine collagen, such as that from Atlantic cod, this band typically appears between 1625 and 1645 cm^{-1} [2, 7, 11]. In our samples, the Amide I band appeared near 1630 cm^{-1} , indicating hydrogen-bond interactions within the collagen backbone. The band position for collagen samples from codfish skin varied by no more than 2 cm^{-1} and showed strong similarity to commercial collagen (CC), with differences in shape and intensity among thermally treated samples (Figure 5).

The Amide II band, originated from N–H bending and C–N stretching vibrations, although less sensitive to secondary structure changes than the Amide I band, still provides important insights into collagen's structural integrity [29]. In complex polypeptide structures like collagen, the Amide II band may appear sharper due to the rigidity and high order of the THS, being thus associated

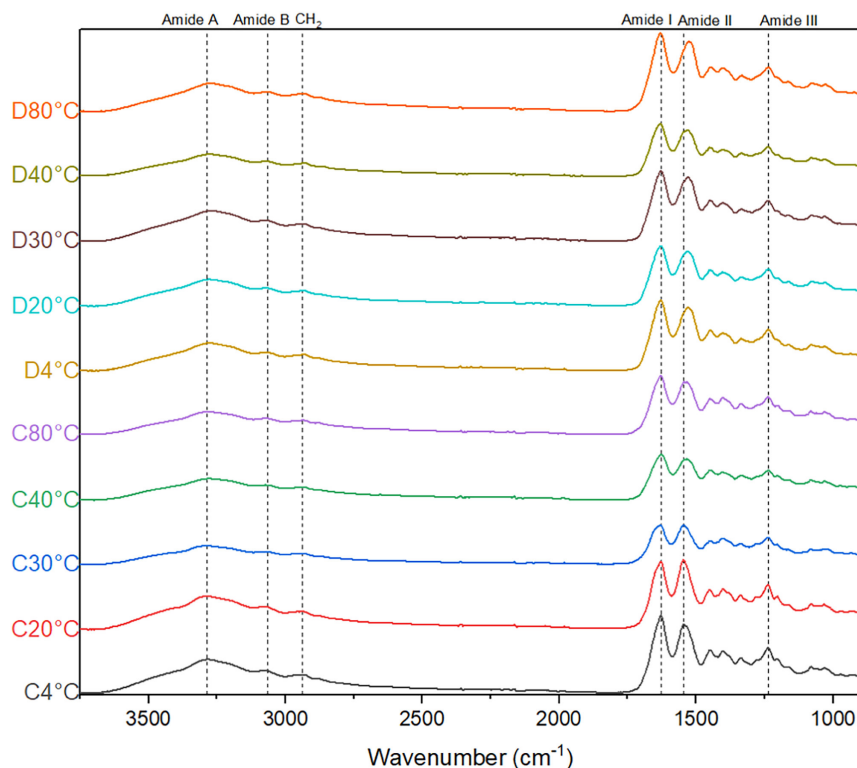


FIGURE 5 | FTIR spectra of the collagen samples, covering the characteristic amide bands between 900 and 3800 cm^{-1} . C: commercial collagen (rat tail Type I collagen). D: collagen extracted from cod fish skin by DES.

with collagen cross-links. Moreover, its intensity may vary slightly depending on the extraction process used [29, 51]. Additionally, collagen's hydration level strongly influences the Amide II band, as water content in collagen increases, the number of absorbed water molecules rises leading to a broader band half-width [45]. In our study, the Amide II band absorption peaks for codfish collagens were observed at lower frequency (17 cm^{-1}) than that of commercial collagen, which appeared at 1525 cm^{-1} (Figure 5). This shift may be attributed to the extraction process, although the observed structural features remain valid and consistent with previously reported data for marine collagen [11, 23].

The Amide III band, originating from N–H bending and C–N stretching vibrations, serves as a key indicator of collagen's helical integrity and is particularly sensitive to structural changes like proline hydroxylation. Furthermore, its position is influenced by peptide backbone bending and stretching vibrations, hydrogen bonding patterns, and the N–H group orientation relative to the C–N bond [37]. For collagen, the Amide III band is typically observed near 1245 cm^{-1} , indicating the presence of a triple-helical conformation and relatively weaker hydrogen bonding [29, 37]. In more tightly packed structures, stronger vibrational coupling may result in a sharper Amide III band; therefore, collagen shows reduced vibrational coupling between adjacent peptide bonds compared to α -helices and β -sheets [37, 52]. The intensity of this band also reflects the hydroxyproline content, which contributes to helix stability of collagen via hydrogen bonding and is closely associated with the Amide III band characteristics [53]. In denatured collagen, the overall appearance of the Amide III band, typically consisting of three bands near 1280 , 1230 , and 1200 cm^{-1} , is weaker, broader, and shifted in frequency. These changes are indicative of a transition to random coil structures and a loss of helical structure integrity [29]. In our study, the Amide III region for collagen obtained from codfish skin by DES displayed three components near 1280 , 1235 , and 1203 cm^{-1} , consistent with the commercial collagen sample; however, the central peak was observed at a slightly lower frequency (3 cm^{-1}) (Figure 7).

FTIR analysis revealed that all samples retained a well-defined Amide III band at positions comparable to commercial collagen, except for those treated at $\geq 40^\circ\text{C}$. Collagen samples exposed to 80°C exhibited marked changes in Amide III band intensity and shape comparing to the samples at 4°C , suggesting disruption of the native THS. To quantify this effect, the standard Amide III/ 1450 cm^{-1} ratio was calculated as an indicator of collagen degradation [11, 54]. The absorption ratios between the Amide III (1230) and 1450 cm^{-1} bands was ≥ 1.00 across all samples except for D 30°C and D 80°C (Figure 6 and Table 2), which denotes the state of the triple helical structure in collagen samples [11, 54]. Amide III, 1230 – 1450 cm^{-1} bands, absorbance ratio is accepted as a key indicator of triple helix structural integrity in collagen, although its consistency of the data with other analysis will be further addressed in the Discussion section.

3.5.2 | Other Characteristic Bands

As marine collagen undergoes hydrolysis, FTIR analysis shows a decrease in the intensity of the Amide I and II bands, alongside the appearance of new bands related to free amino groups, typically observed at approximately 1440 cm^{-1} [43, 53]. In our study, a band near 1450 cm^{-1} was present in all collagen samples, with differences in band shape observed in codfish collagen samples against the commercial collagen (Figure 7). The band near 1335 cm^{-1} , representing hydroxyproline content and cross-linking density, appeared at the same position across samples but exhibited differences in shape and intensity, being less sharp in codfish samples when compared with rat tail collagen, reflecting the lower cross-linking typically found in marine collagen [26]. A peak near 1080 cm^{-1} was also observed across all collagen samples (with various band shapes and intensities) which indicates the presence of fibrillar collagen, with its intensity representing the degree of fibrillation [51]. Moreover, another peak near 1030 cm^{-1} was observed for all collagen samples, which indicates C–OH stretching vibrations linked to carbohydrate groups on the protein, while bands at 1450 , 1400 , and 1335 cm^{-1} correspond to CH_2 and CH_3 wagging, deformation, and CN stretching in collagen (Figure 7) [29].

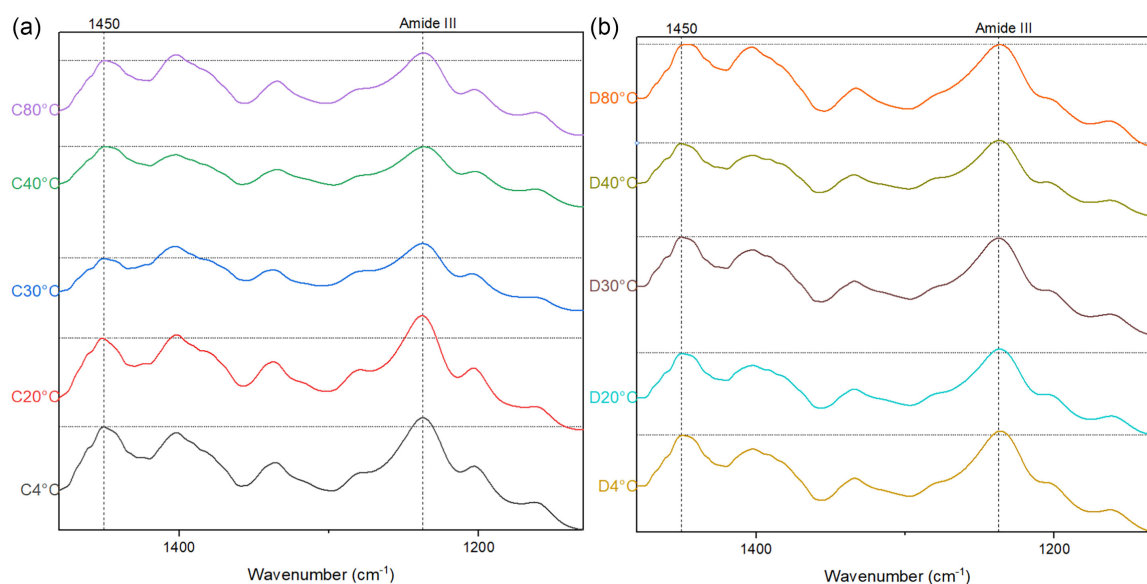


FIGURE 6 | Amide III/ 1450 cm^{-1} absorbance value. (a) Commercial collagen (rat tail Type I collagen [C]). (b) Collagen extracted from cod fish skin by DES (D).

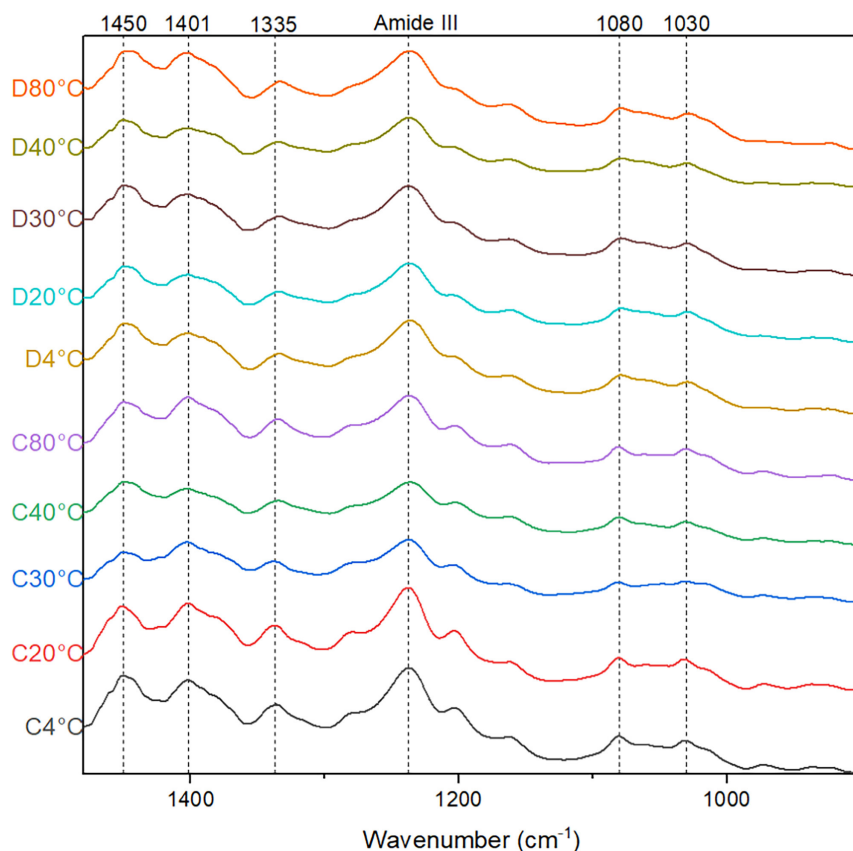


FIGURE 7 | FTIR spectra of the collagen samples, covering bands at the range from 900 to 1500 cm^{-1} . C: commercial collagen (rat tail Type I collagen). D: fish skin collagen extracted by DES.

3.6 | Collagen Structural Integrity

Collagen of mammalian origin has a higher degree of cross-linking, which confers it a higher structural integrity and thermal stability, as evidenced by a higher denaturation temperature [55]. The higher cross-linking in mammalian collagen is mainly attributed to enzymatic covalent cross-links involving lysine and hydroxylysine residues during tissue maturation, while proline and hydroxyproline primarily contribute to triple helix stability rather than intermolecular cross-linking [56]. These properties are reflected in the positions and shapes of the Amide I, II and III bands in FTIR spectra [37]. The lower degree of cross-linking of codfish collagen results in such differences [29, 57]. Additionally, variations in band patterns observed in collagens derived from fish in warmer habitats highlight how FTIR characteristics can differ depending on species and environmental factors. These results confirm the complexity of interpreting FTIR data in collagen analysis [41]. Marine collagen typically shows a single, broad Amide I peak at a lower frequency compared to terrestrial sourced collagen, potentially due to its lower hydroxyproline content [45]. In codfish skin collagen, this lower Amide I frequency may be explained by the specific amino acid composition of the α -chains, particularly the amino acid preceding glycine, which can lower the Amide I band and raise the Amide II band due to stronger intermolecular hydrogen bonding involving the basic imino carbonyl group [42, 45]. Also, a narrow Amide I band at lower frequency generally indicates higher hydration levels within the triple helical structure of collagen [50]. The appearance of a broad peak near 1645 cm^{-1} is often associated with random

coil conformations, suggesting structural disorder [52]. Literature reports that longer chains tend to produce sharper amide bands, whereas broader half-widths above 30 cm^{-1} suggest irreversible unwinding and random coil formation, indicating partial degradation of the extracted collagen [42]. However, these findings were not evident in our analyses.

The Amide I band position of codfish collagen extracted with the DES (D4°C) was closely aligned with the commercial collagen standard (C4°C) (Figure 5), at slightly higher position to that of the commercial (2 cm^{-1}), displaying a narrow and well-defined band shape. The Amide III band also appeared at slightly lower frequency (3 cm^{-1}) positions when compared with commercial collagen, although narrow and well defined, representing the good structural integrity of the collagen sample. Following thermal treatment at 80°C for 72 h, the most pronounced structural changes were observed in samples C80°C and D80°C. Such samples showed modifications in the FTIR spectra, particularly in the Amide III region. These included decreases in band intensity and increased broadening, all indicative of structural changes in the collagen (Figures 5 and 7). The concurrent broadening of this band further reflects increased structural disorder, likely due to disruption of the triple-helical architecture [37, 51, 53]. Due to the complex nature of Amide II band, arising from both N—H deformation and C—N stretching, it is challenging to use it to interpret for the collagen's conformation [45]. Shifts and broadening of this band often point out structural disruption in collagen's triple helix, indicating a loss of organized hydrogen bonding as the structure unwinds. Although spectral shifts alone are not

definitive indicators of degradation, decreased band intensity and increased smearing are commonly observed following denaturation; however, Amide II reflects hydration and cross-linking level by typically shift to higher wavenumbers in hydrated collagen ($1550\text{--}1555\text{ cm}^{-1}$) and to lower values in the dry state (around 1535 cm^{-1}) and shift to lower positions reported to be associated to the extraction process [58, 59]. In our study, the Amide II band absorption peaks for all codfish collagen samples are observed at some 17 cm^{-1} lower frequency than for commercial collagen (Figure 5). The lower Amide II band position observed for marine collagen relative to commercial collagen has also been reported to be associated with collagen destabilization and loss of native THS [60]. The Amide II band of commercial collagen retained both its shape and position at 20°C , remaining identical to that observed at 4°C (1545 cm^{-1}). In contrast, upon thermal treatment at 40°C and 80°C , the Amide II band shifted to lower wavenumbers, reaching 1538 cm^{-1} . A similar trend was observed for codfish skin-derived collagen. The sample treated at 20°C exhibited an Amide II band position and profile comparable to that at 4°C (1529 cm^{-1}), whereas samples treated at 80°C showed progressive downshift (Figure 5). The Amide II band shift to lower frequency indicating a temperature-dependent effect supports the conclusion that the downshift of the Amide II band toward lower wavenumbers is primarily a consequence of thermal destabilization and partial loss of collagen's native structure reported by Madaghiele et al. [60], rather than an artifact arising from the extraction process itself [58, 59].

The CD analysis showed that the characteristic positive band at $\sim 220\text{--}222\text{ nm}$, indicative of an intact triple helix, is preserved in commercial collagen with only a slight decrease from 4°C to 20°C , whereas this band is absent in codfish collagen, indicating partial disruption of the triple helical structure, likely due to biomass condition and/or extraction conditions. Nevertheless, the negative band ($\sim 199\text{ nm}$) in codfish collagen remains relatively unchanged between 4°C and 20°C , suggesting no further structural alteration within this temperature range (Figure 4b). XRD analysis indicated that the fibrillar organization is maintained at lower temperatures and only disrupted at elevated temperatures (Figure 3). These findings highlight that, despite partial loss of triple helical order, the supramolecular fibrillar structure of codfish collagen remains stable under mild thermal conditions. In addition, a quantitative comparison of the area under curve (AUC) analysis of UV-Vis $200\text{--}250\text{ nm}$ (Table 2) demonstrates a clear two-stage thermal profile showing that codfish skin collagen maintained its native THS at 20°C . For the codfish collagen, a stability plateau exists between 4°C and 20°C (AUC variance $<0.2\%$), followed by a measurable hyperchromic shift starting at 30°C (87.94) and peaking at 80°C (88.87) (Table 2). Collectively, these results demonstrated that aqueous DES extraction at ambient temperature can be considered a nondestructive, energy-efficient process compared with conventional extraction at 4°C , yielding high-quality Type I collagen without significant alteration of its structural integrity in codfish skin and related marine biomass, supporting its potential for large-scale production.

3.7 | Reassessment of Amide B Band

Many studies on marine-sourced collagen report and discuss the Amide B band near 3070 cm^{-1} [46, 48, 61, 62], while other

works report a broader range ($2920\text{--}3100\text{ cm}^{-1}$), which may vary depending on the extraction method used [29]. Doyle et al. [45] and Cortizas et al. [57] identified the band near 3070 cm^{-1} as reflecting the N–H stretching and considered it part of the Amide A band. Barth et al. [63] reported that the Amide A band typically forms part of a Fermi resonance doublet, with Amide B appearing as a weaker component between 3100 and 3030 cm^{-1} . In contrast, others point to this band near 2930 cm^{-1} , attributing it to CH_2 asymmetric stretching vibration [64–67]. Si et al. [53] reported Amide B band closer to 3076 or 3080 cm^{-1} , linking it specifically to asymmetric CH_2 stretching vibrations, while Barzideh et al. [68] claimed that asymmetrical stretch of CH_2 and NH_3^+ and the explanation for observing the Amide B band at a higher frequency are both attributed to the presence of the free NH_3^+ group of lysine. These discrepancies may result from different experimental conditions, sample origin, or variations in protein secondary structure (Table 3). However, Cortizas et al. [57] further assigns bands within the $2800\text{--}3050\text{ cm}^{-1}$ range to lipid and aliphatic content, while Muyonga et al. [69] assigned the band near 2924 cm^{-1} to CH_2 asymmetric stretching. Similarly, Yongjie et al. [70] and Ampitiya et al. [71] observed Amide B bands below 2940 cm^{-1} , which they attributed to aliphatic moieties rather than peptide backbone vibrations. Our observations of the samples after thermal treatment are consistent with the results of Cortizas et al. [57], who attributed the band near 2930 cm^{-1} to the presence of aliphatic compounds rather than to a true amide. Likewise, our data align with Lazarev et al. [44] and Doyle et al. [45], who report that the Amide B band typically appears near 3070 cm^{-1} , associated with N–H stretching vibrations and often considered part of the Amide A region. Since amide bands in marine-derived collagen generally appear at lower wavenumbers, the peak near 2950 cm^{-1} , commonly misassigned to Amide B, is more likely due to CH_2 stretching vibrations of aliphatic compounds. A true Amide B resonance should appear above 2950 cm^{-1} , as a shoulder of the Amide A band. Therefore, bands observed below this threshold likely represent overlapping NH and CH stretching contributions from residual aliphatic moieties rather than peptide vibrations backbone. Such spectral misassignments may arise from the inadequate sample pretreatment and/or the presence of impurities, more specifically residual aliphatic compounds such as hydrocarbons, in the collagen extracts.

3.8 | Reassessment of the Absorbance Intensity Ratio of Amide III/ 1450 cm^{-1}

The absorption intensity ratio of the Amide III band to the 1450 cm^{-1} band is used as an indicator of the triple-helical integrity in collagen samples [11, 54] (Table 3). A ratio near to 0.5 reported to be associated with disrupted or denatured collagen, while values closer to 1 have been reported in samples retaining their native triple helical structure and a ratio near 0.84 has been found in gelatin, compared to higher values in native Type I collagen [11, 72, 73]. In our study, all samples exhibited ratios ≥ 0.99 after thermal treatment (base line corrected and normalized, Figure S1 and Table 2). For commercial collagen specifically, the ratio varied from 1.04 to 1.1 and back to 1.04 upon heating from 4°C to 80°C . In codfish collagen sample extracted using DES, the ratio remained ≥ 0.99 , with maximum variation upon heating not exceeding 0.02 (Table 2), which is much higher than 0.50, or even 0.85. However, complementary analyses clearly demonstrate

TABLE 3 | Comparative analysis of extraction conditions and physicochemical characteristics (FTIR, UV, XRD, CD, and SDS-PAGE) across diverse marine biomasses.

Biomass	Extraction temperature, °C	UV-Vis, nm	SDS-PAGE, kDa			XRD, 2θ	CD, mdeg			FTIR amide bands, cm ⁻¹			Amide III/1450cm ⁻¹ ratio	Ref.	
			α1	α2	β		+222	-200	A	B	I	II			III
Salted skin Atlantic cod (Gadus morhua)	4	220	115	230	8.1°	Not detected	Detected	3270	3080	1630	1525	1230	1.04	This work	
<i>Prionace glauca</i> , <i>Zearaja chilensis</i> , and <i>Bathyraja brachyurops</i> cartilages	4	230	130	250	NA	Detected	Detected	3270	3080	1630	1580	1235	Close to 1	[74]	
Skin of red snapper	4	NA	130	250	NA	Detected	Detected	3330	3080	1630	1550	1238	1.02	[75]	
Salt-cured Atlantic cod (Gadus morhua) skin	4	231	120	245	7°	NA	NA	3194	2882	1634	1532	1238	0.95	[67]	
Fresh codfish skins	4	NA	130	250	NA	Not detected	Detected	3320	2880	1640	1554	1239	Close to 1	[2]	
Marine sponges (<i>Chondrilla caribensis</i> and <i>Aplysina fulva</i>)	2–5	NA	ND	ND	NA	Not detected	Detected	3270	ND	1640	1522	1230	NA	[76]	
Salt-cured Atlantic cod (Gadus morhua) skin	37 (3 h, at 37°C, in a humidified 5% CO ₂)	NA	125	245	12°	Detected	Detected	3200	2953	1635	1543	1240	1.01	[66]	
Bigeye tuna (<i>Thunnus obesus</i>), bone, skin with scales	<4	NA	125	250	NA	NA	NA	3301	2927	1639	1546	1240	Around 1	[11]	
Preserved salted codfish skins	4	NA	130	250	7.6°	Detected	Detected	3288	2926	1633	1535	1240	Close to 1	—	
Preserved salted codfish skins	4	230	125	250	12°	Not detected	Detected	3392	2924	1635	1537	1236	NA	[7]	
Tarakihi (<i>Nemadactylus macropterus</i>) skin	4	NA	120	245	NA	Detected	Detected	3300	2920	1640	1540	1235	0.85	[77]	
Pacific saury scale	4	NA	116	220	NA	NA	NA	3306	2920	1630	1540	1235	1.03 (reported as denatured collagen)	[48]	

Abbreviation: NA, not available.

that the structural integrity of collagen in the higher temperature samples is markedly different. Our results show a clear decrease in Amide III absorbance intensity and pronounced alterations in band shape (across all three components) at temperatures above 40°C, indicating disruption of collagen structural integrity and loss of its native state. Because thermal treatment causes a concurrent decrease in the absorbance intensities of both the Amide III and 1450 cm⁻¹ bands, their ratio may remain close to unity, even when the collagen structure has been substantially degraded at elevated temperatures, as it can be seen in Table 2.

While FTIR provides valuable insights into the structural integrity of marine-derived collagen, reliance on amide band intensity ratios alone may be misleading. Thus, FTIR analysis should be complemented by electrophoretic analysis (SDS-PAGE) and XRD to assess crystalline and fibrillar organization and CD spectroscopy to characterize collagen secondary structure. Combining these techniques delivers a more robust and comprehensive characterization of collagen's molecular structure. As shown in Table 2, the ratio of absorbance unit of Amide III/1450 cm⁻¹ for commercial collagen remained close to 1.00 (0.99) even after thermal treatment at 80°C for 72 h. This value may imply that the collagen maintained its native structure; however, results from XRD and CD clearly demonstrated substantial changes in the triple-helical conformation and altered molecular order, indicating that structural integrity was not similar relative to the sample subjected to 4°C. Similarly, DES-extracted codfish collagen treated at 30°C displayed an XRD peak near $2\theta \approx 8.7^\circ$, yet the ratio of absorbance unit of Amide III/1450 cm⁻¹ was 0.99 equal to that of the 80°C sample. Based on this ratio alone, both samples would have similar THS structure, despite XRD and CD showing clear differences in structural preservation. These results demonstrate that the Amide III/1450 cm⁻¹ threshold is insufficient as a standalone indicator to assess the collagen structural integrity. Furthermore, the absolute θ_{222} and θ_{199} ellipticity values obtained from CD provide an additional complementary indicator of structural degradation, particularly for the commercial collagen treated at 80°C and the codfish collagen samples treated at 40°C and 80°C, both bands decreased markedly. In such cases (C80°C and D80°C), the absolute ellipticity decay and such behavior were fully concordant with SDS-PAGE, XRD, and FTIR results, reinforcing the conclusion that collagen structural integrity can only be accurately assessed through integrated multitechnique analysis (Table 2).

The FTIR second-derivative spectra provided further insight into the stability of the Amide III/1450 cm⁻¹ ratio, which remained close to unity across the investigated temperature range (Figure S4). Although fluctuations in the intensity of the band near 1457 cm⁻¹ were observed under different thermal conditions, these changes lacked a systematic trend (increase or decrease) from 4°C to 80°C. This suggests that thermal stress influences the local environment of the pyrrolidine rings, but the Amide III/1450 cm⁻¹ ratio alone cannot be considered a definitive marker of triple-helix stability in this system, where structural transitions are expected. This limitation is further supported by the behavior of the Amide I and II bands. The Amide II band showed a pronounced red shift to lower wavenumbers, accompanied by a marked decrease in the intensity of both Amide I and II as the temperature increased toward 80°C (Figure 5). The results of this study highlight important limitations of this approach. This

indicates that such values do not simply reflect preservation of the triple helical structure when considered in isolation. Therefore, it should not be used as a standalone indicator of collagen structural integrity. Reliable interpretation requires complementary analyses, such as CD and XRD, which probe different hierarchical levels of collagen structure.

4 | Conclusions

In this study, we employed multiple analytical techniques to investigate the thermal stability of Type I collagen extracted from salted codfish skins. The extraction was performed under a single controlled condition (4°C) to decouple extraction-induced structural changes from thermal effects. Samples were subjected to thermal treatment from 20°C to 80°C for 72 h. UV-Vis spectroscopy, SDS-PAGE, XRD diffraction, CD spectra, and FTIR spectra confirmed a similar triple-helical conformation for collagen samples at 4°C–20°C. In contrast, higher temperatures led to significant collagen structural alteration. We also reassessed the commonly used Amide III/1450 cm⁻¹ absorbance ratio for Type I collagen. This ratio cannot be considered a standalone indicator of collagen structural integrity, as values close to unity may persist even after significant structural degradation. Exposure to elevated temperatures, particularly at 80°C, resulted not only in collagen unfolding but also in significant hydrolytic degradation. This study demonstrates that the stringent requirement for 4°C extraction is not essential for preserving the structural integrity of collagen from salted codfish skin and similar marine biomass, and extraction at controlled ambient temperatures yields a product of equivalent purity and stability. These findings offer a more scalable and sustainable framework for marine-biomass derived collagen production, effectively eliminating the need for energy-intensive cooling without compromising quality, suggesting feasibility of Type I collagen extraction from salted codfish skin and related marine biomass.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.