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ORIGINAL PAPER

Collagen from Atlantic cod (*Gadus morhua*) skins extracted using CO_2 acidified water with potential application in healthcare

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Abstract

The extraction of collagen from fish skins is being proposed as strategy for valorization of marine origin by-products, being a sustainable alternative to mammal collagen. The method commonly uses solutions of organic acids, but new methodologies are arising, aiming to improve process yields and/or the properties of the resulting products. In this work, skins removed from salt brine Atlantic cod (*Gadus morhua*) were used to extract collagen, using water acidified with CO_2, obtaining an extraction yield of 13.8% (*w*/w). Acidified water extracted collagen (AWC) presented a total content of proline-like amino acids of 151/1000 residues, with a degree of hydroxylation of 38%, and its SDS-PAGE profile is compatible with type I collagen. Moreover, FTIR, CD and XRD results suggest the presence of preserved triple helix, having a denaturation temperature of 32.3 °C as determined by micro-DSC. AWC exhibited a typical shear thinning behavior, interesting regarding their further processing, namely in jelly-like formulations. Additionally, the presence of AWC in MRC-5 human fibroblasts culture did not affect cell viability, demonstrating the non-cytotoxic behavior. Overall, the results support the efficiency of the proposed approach for collagen extraction and further enable the design of methodologies to address AWC use in biomedical or cosmetic context.

Keywords Fish collagen · Cod skins · Green extraction · CO_2 acidified water · Supercritical fluids · Marine biomaterials

Introduction

Atlantic cod (*Gadus morhua*) is a central player in the economy of countries like Norway and Iceland [1], being processed in high quantities for human consumption, which generates as well a very significant amount of by-products, as skins, bones and viscera [2]. Strategies for the valorization of these marine by-products are being studied, not only to improve the sustainability of this fish species, but also as an added-value waste treatment procedure, with ecological and economic advantages [3, 4]. Equally important and regarding their great biotechnological potential, cod by-products can be established as alternative raw-materials for the production of biomolecules highly demanded by several industrial sectors. In particular, cod skin is a significant by-product generated when cod is processed as fillets and/or shredded, which could be utilized to produce collagen [5–8], often incorporated in the production of functional food or medicine due to its properties.

Collagen represents the main structural protein of the various connective tissues in animals, accounting for approximately 30% of all vertebrate body protein [9–11]. In particular, marine-origin collagen from fish skin, scales and bones have been reported in the literature, representing a relevant path to explore, given that the current industrial demand for collagen accounts up to 326,000 tons for different fields of application including food, cosmetics, pharmaceutical and biomedical [10, 12, 13]. Studies show that collagen can be

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isolated after acid, basic or enzymatic treatments, requiring the use of significant amount of water; nonetheless, the extraction procedures described are very laborious and involve several and time-consuming steps, which holdup the scaling up of the processes. The final characteristics of the product depend not only on their source (fish by-product) but also on the extraction methodologies used. The use of volatile organic solvents has been decreasing due to strict legislation and environmental concerns forcing industries to move forward the application to alternative processing methods, which comply with the green philosophy and essential for their competitiveness [14]. In that sense, new procedures are being studied in order to comply with the principles of green chemistry, and environmentally friendly alternative processes based in alternative technologies are reported in the literature. This is for example the case of the extraction by supercritical fluids technology or the use of water acidified with carbon dioxide. Carbon dioxide is the most widely used candidate in this technology due to its attractive properties such as low toxicity, flammability and cost, as well as high availability, stability and environmental acceptability, but also due to its operating conditions at moderate temperatures and pressure values. Additionally, carbon dioxide is used as a reversible acidifying agent, which is released from the aqueous media after extraction and hence obtaining a purified compound [15, 16]. The use of water acidified with CO_2 comprises one single extraction step with soft conditions of operation and avoids the use of any organic solvent [17].

The main objective of the present study is the valorization of cod skin resulting from the processing of this fish for food purposes and the establishment of a new extraction process, using water and carbon dioxide (CO_2) as solvents with a natural origin raw material to obtain collagen. An extensive physical-chemical characterization was carried out, as well as the analysis of the cytotoxicity and the rheological behavior of the isolated collagen with a view to the future application in cosmetics and biomedical context.

Materials and methods

Materials

Atlantic cod (*Gadus morhua*) skins were kindly offered by Frigoríficos da Ermida, Lda. (Gafanha da Nazaré, Portugal), after being removed from frozen specimens (that have been previously preserved in salt brine), and stored at −20 °C until use. Samples of acid soluble collagen (ASCs) from the same batch of skins were obtained under other studies in the lab following a standard acetic acid extraction procedure [5] and used as reference material. All other reagents were of analytical grade and used as received.

Extraction of collagen

The extraction of collagen from cod skins was achieved using supercritical fluids technology, in particular with water acidified by pressurization with CO_2, similar to the methodology previously used for extraction of collagen from marine sponges [14, 17, 18]. The skins were cleaned several times with distilled water, for removal of muscle debris, scales and salt, and further grounded in small pieces. The wet skin was weighed and placed in a high-pressure vessel (30 cm^3) with distilled water (1 g per 20 ml). The vessel was heated to 37 °C, with the system being then pressurized with carbon dioxide up to 50 bars. The extraction was performed in batch mode (discontinuous mode, without exchanges with the outside) for 3 h. After, the high-pressure vessel was rapidly depressurized and the extract was obtained by filtering twice, with filter paper followed by a second filtration with a 0.45 μm syringe filter. The retrieved acidified water extracted collagen (AWC) was frozen, freeze-dried and stored at room temperature until further use.

The extraction yield was determined as the ratio between the produced AWC and the initial wet weight of skin biomass (Eq. (1)).

$$\textbf{Yield of collagen (wet)(\%)} = \frac{\text{Weight of collagen (g)}}{\text{Weight of wet skin (g)}} \times \mathbf{100} \quad (1)$$

Characterization of the extracted collagen

Fourier-transform infrared (FTIR) spectroscopy

The infrared spectra of collagen samples were obtained with a Shimadzu- IR Prestige 21 spectrometer in the spectral region of 4000–800 cm^{-1}, with resolution of 2 cm^{-1}, as average of 32 scans. The samples of AWC and ASC were mixed with potassium bromide (KBr) and pellets were obtained using a hand press, which were analyzed in transmission mode.

SDS-PAGE analysis

Dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was prepared using reagents from Sigma SDS-PAGE reagents and casted on a Biorad Mini Protean II System. Freeze-dried collagen samples were dissolved in 0.5 M acetic acid (2 mg/mL) under stirring. The solutions were then mixed 1:1 (*v*/v) with loading buffer and heated 5 min at 65 °C and 10 min at 95 °C to completely denature the proteins. The collagen samples (20 μL) were loaded onto SDS-Page gel as well as 4 μL of protein marker. Both separating and stacking gels were run at 90 V. After running, the gels were stained in a

Coomassie (0.500 g Coomassie Brilliant Blue G-250 (Biorad), 500 mL methanol, 100 mL acetic acid and 400 mL deionized water) staining solution for 30 min. Excess stain was removed with Destain I (80 mL methanol, 14 mL acetic acid and 156 mL deionized water) for 30 min and Destain II (12.5 mL methanol, 17.5 mL acetic acid and 220 mL deionized water) overnight, under stirring, until background removal of the stained solution. These destain steps enable the properly visualization of collagen profiles.

Amino acid analysis

The amino acid content of collagen samples was determined by quantitative analysis using a Biochrome 30 amino acid analyzer (Biochrome Ltd., Cambridge, U.K.). Briefly, the samples were completely hydrolyzed and separated by an ion Exchange column. After post-column derivatization by ninhydrin, the collagen samples were analyzed at two wavelengths: 440 and 570 nm. An internal standard of norleucine was used to determine the concentration of amino acids in the sample.

Micro differential scanning calorimetry

Micro-DSC measurements were carried out with a high-sensitivity SETARAM Micro DSC III microcalorimeter by using 700 μL samples in stainless steel cells to determine the denaturation temperature of collagen. All collagen samples were dissolved at 5 mg/mL in 0.5 M acetic acid, under stirring, at 4 °C. The mass of sample was weighed directly in the measure cell and 0.5 M acetic acid solution was used in the reference cell during DSC scans. Measurements were made at 1 K/min scan rate in the temperature range of 5–80 °C. Nitrogen was used as purge gas to avoid humidity condensation on the circulating coils of the microcalorimeter.

Circular dichroism analysis

Circular dichroism (CD) measurements were performed using a Jasco Model J1500 spectropolarimeter (Jasco, U.K.) using a quartz cylindrical cuvette (Hellma, Germany) with a path length of 2 mm. The cuvette was filled with 600 μL of 0.1 mg/mL AWC solution in 5 mM sodium phosphate, at pH 4, and the CD spectra were obtained by continuous wavelength scans (average of three scans) from 180 to 240 nm at a scan-rate of 50 nm/min, at 4 °C.

X-ray diffraction (XRD)

XRD measurements were obtained using a conventional Bragg–Brentano diffractometer (Bruker D8 Advance DaVinci, Germany) equipped with CuKα radiation, produced at 40 kV and 40 mA. Data sets were collected in the 2θ range of 5–40° with a step size of 0.02° and 1 s for each step. The average crystallite size was estimated with the Bragg equation (Eq. (2)): $\mathbf{d\ (Å)} = \lambda/2\sin\theta$ (λCukα1 = 1.5406).

Gel permeation chromatography – size exclusion chromatography

The studied collagen sample (AWC) was analyzed by size exclusion chromatography with a refractometer, right angle laser-light scattering and viscosity detectors (TDA 305 – Viskotec, Malvern Instruments, UK). Novema Precolumn (10 μm, 8 × 50 mm), Novema 30 Å (10 μm, 8 × 300 mm), Novema 1000 Å (10 μm, 8 × 300 mm) and Novema 1000 Å (10 μm, 8 × 300 mm) were used for the analysis. The system was kept at 30 °C and 0.15 M NH_4OAc/0.2 M AcOH buffer (pH 4.5) was used as eluent, at rate of 1 mL/min. Pullulan 47 kDa and polydispersity index (PDI) 1.07 (PSS standard services) was used to obtain a multidetector calibration (used to obtain the absolute Mw). The dn/dc was established as 0.186 after a literature search [19].

Rheology

Rheological analyses were performed using a Kinexus pro+ rheometer (Malvern Instruments, UK), with acquisition software rSpace. The measuring system used in these experiments was composed by a stainless steel cone (40 mm of diameter and 4°) and plate geometries. The surface geometry was covered with dodecane to prevent water loss. Rotational experiments were performed in order to obtain shear viscosity as a function of the shear rate, from 0.1–1 to 1000 s^{-1}, at 25 °C. All plots were obtained from the average of 3 experiments. These experiments were conducted with 3% (*w*/*v*) AWC solution in 0.02 M acetic acid.

Cytotoxicity

Cell expansion Human fibroblast cell line (MRC-5) was cultured in D-MEM low glucose medium (Sigma-Aldrich) supplemented with 10% FBS (Alfagene) and Pen/Strep (100 U/100 g/mL; Life Technologies). Fibroblasts were used at passages 17–22. Cells were incubated at 37 °C in a humidified 5% CO_2 atmosphere. Media was exchanged every 2–3 days until cells reached a 90% confluence.

Cell culture Cells were harvested and 15,000 cells were cultured in 24 well-plates. The cells were left to adhere for 4 h and, after that, AWC extracts were added to adherent cells. Extracts were dissolved in the culture medium at different concentrations: 0.25, 0.5, 1, 2, 3, 4, and 5 mg/mL. For all the assays a control was used (no biological extract in the culture medium). Each experimental condition was tested in triplicate and two independent assays were performed.

Cell viability The metabolic activity of cells, cultured with different extracts concentrations and time points, was determined by the MTS assay (CellTiter 96 AQueous One Solution, Promega), in which, basically, the quantity of formazan product is directly proportional to the number of living cells in culture. At days 1, 2, and 3, the culture medium was removed and the testing conditions were rinsed with sterile Phosphate-Buffered Saline (PBS). A mixture of culture medium (without FBS and phenol red) and MTS reagent (5:1 ratio) was added to each well and left to incubate for 3 h, at 37 °C, in a humidified 5% CO_2 atmosphere. Thereafter, the absorbance of the MTS reaction medium from each sample was read in triplicate at 490 nm in a microplate reader (Synergy HT, Bio-TEK). All experiments were performed in triplicate.

Statistical analysis

All data values are presented as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism Software. Differences between the different conditions of the cellular assays were analyzed using nonparametric test (Kruskal–Wallis test) and $p < 0.05$ was considered significant.

Results and discussion

Extraction of collagen

Collagen has been isolated from fish skins from different species using acid extraction, commonly based in acetic acid solution, although solutions of other acids have been used as well. In this work, a different strategy has been used, with the acidic solution being produced by pressurizing water with CO_2 (Fig. 1), used as media for the extraction of cod collagen. Using this methodology, an extraction yield of 13.8% ± 0.013, significantly higher than the one obtained with previous studies using the conventional methodologies based in acetic acid solutions, namely the ones reported by Alves et al. [5] and Sousa et al. [20] in which acid soluble collagen from cod by-products was extracted with 0.5 M acetic acid solution. This yield improvement, may be due to the fact that the process proposed in the present study consists of a single step with mild conditions and using acidified and pressurized water as the only solvent, although the co-extraction of gelatin could not be excluded as also observed when a similar CO_2 acidified water methodology was used for the collagen extraction from marine sponges [14, 17].

SDS-PAGE analysis

The protein components of AWC extract was assessed by SDS-PAGE (Fig. 2), together with ASC and type I collagen

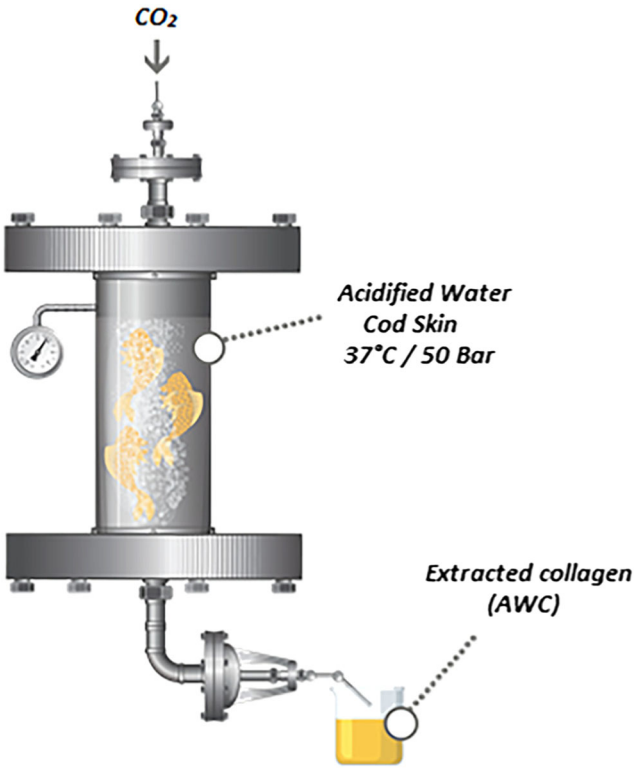

Fig. 1 Schematic representation of the AWC extraction process

from bovine skin, used as reference samples. The electrophoretic profile of AWC is similar to the one obtained for ASC and bovine type I collagen, with all exhibiting the characteristic alpha 1 and alpha 2 bands, with the former being more intense than the latter, compatible to the fact that each type I

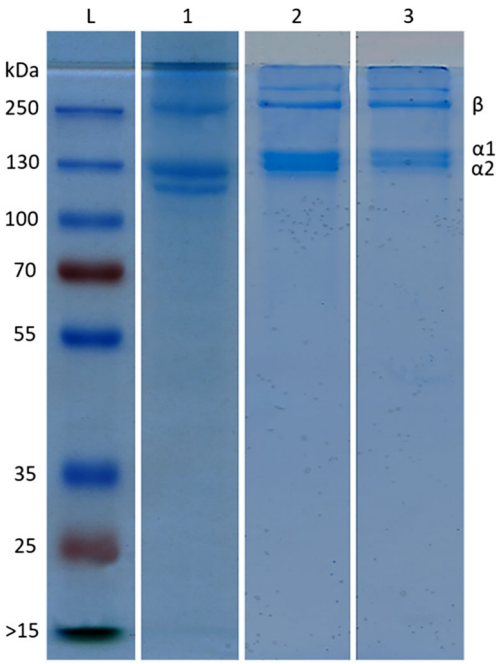

Fig. 2 SDS-PAGE patterns of a) protein marker, b) collagen from bovine skin, c) ASCs and d) AWC from cod skin

collagen molecule is composed by two alpha 1 chains and one alpha 2 chain. Beta dimmers are also visible, due to the presence of associated protein (alpha) chains [10, 20].

Nevertheless, the alpha bands in the marine samples are depicted at lower heights, correspondent to smaller molecular weights, which might indicate that marine collagen molecules should be slightly shorter than the ones from bovine skin. When comparing both marine samples, no differences are observed in the bands attributed to collagen, from which one can conclude that the extraction methodology can be a promising alternative to traditional methods. Additionally, the AWC profile (Fig. 2d) contained as well other blurred bands that may be due to impurities or slight denaturation of collagen resulting from the extraction process, as eventually gelatin and collagen protein hydrolysates. This can contribute to the higher extraction yield obtained with the present green methodology.

FTIR analysis

The AWC was characterized by FTIR, and compared with ASC as shown in Fig. 3. Despite the different methods of extraction, the spectra demonstrate similar chemical structure, suggesting that the extracts present similar chemical compositions. The spectrum of AWC exhibited the characteristic peaks of Amide A, Amide B, Amide I, Amide II and Amide III observed for collagen samples. The Amide A band, observed in a range between 3200 and 3500 cm^{-1}, is relative to the N-H stretching vibration typical of intermolecular hydrogen bonding. In case of Amide B the absorption was found at 2953 cm^{-1} and is associated to the asymmetrical and symmetrical stretch of CH_2. The stretching vibrations of C=O groups of proteins are representative of Amide I peak and occurred at 1635 cm^{-1}. Amide II bands arose at 1543 cm^{-1}, correspondent to NH bending vibration couplet with CN stretching, at 1450 cm^{-1}, attributed to CH_2 bending, and at 1398 cm^{-1}, deriving from COO^- symmetrical stretching. Amide III bands occurred at 1338 cm^{-1}, correspondent to NH bending associated with CN stretching, and at 1240 cm^{-1}, relative to C-O stretching. If the absorption ratio between the amide III and 1450 cm^{-1} peaks is equal to 1, that is, according to Wang et al. [21], an indicator of the preservation of triple helix structure of collagen. The AWC spectrum presented an absorption ratio between the amide III and 1450 cm^{-1} peaks of 1.01, thus suggesting the presence of collagen with the native structural organization.

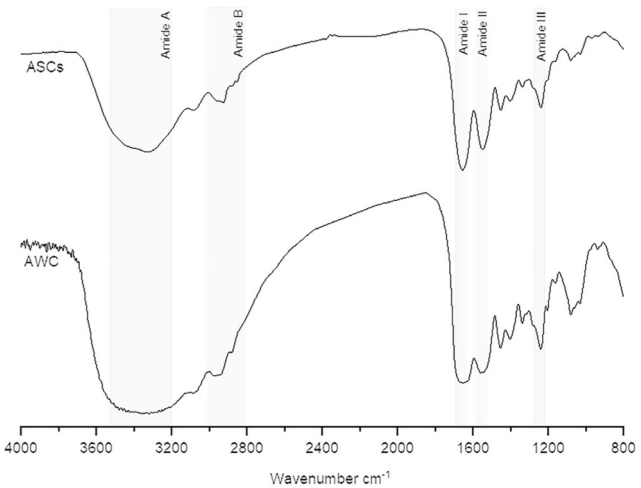

Fig. 3 FTIR spectra of ASC and AWC from cod skin

Amino acid content of collagen extract

The quantitative composition of amino acids in the produced collagen samples was determined and compared to the one obtained for collagen extracted using the conventional methodology and the respective results are depicted in Table 1. The amino acids profiles obtained for AWC and ASC are quite similar, showing that the extracts were rich in glycine (Gly), alanine (Ala), proline (Pro) and hydroxyproline (OhPro), which are characteristic of typical molecules of collagen protein. The major amino acid in AWC is glycine with 345 residues per 1000 residues, followed by alanine with 118. This is consistent with the results found in literature for collagen, namely with the fact that glycine occurs regularly at every

Table 1 Amino acid composition of ASC and AWC from cod skin

Amino acid	ASC	AWC
	Residues/1000 total amino acid residues	
Asp	49	49
Thr	22	23
Ser	68	69
Glu	74	73
Gly	348	345
Ala	121	118
Cys	3	2
Val	15	14
Met	22	18
Ile	8	7
Leu	21	19
Nleu	13	12
Tyr	3	2
Phe	16	11
OhLys	1	5
His	7	7
Lys	27	26
Arg	45	47
OhPro	50	58
Pro	89	94
Total	1000	1000

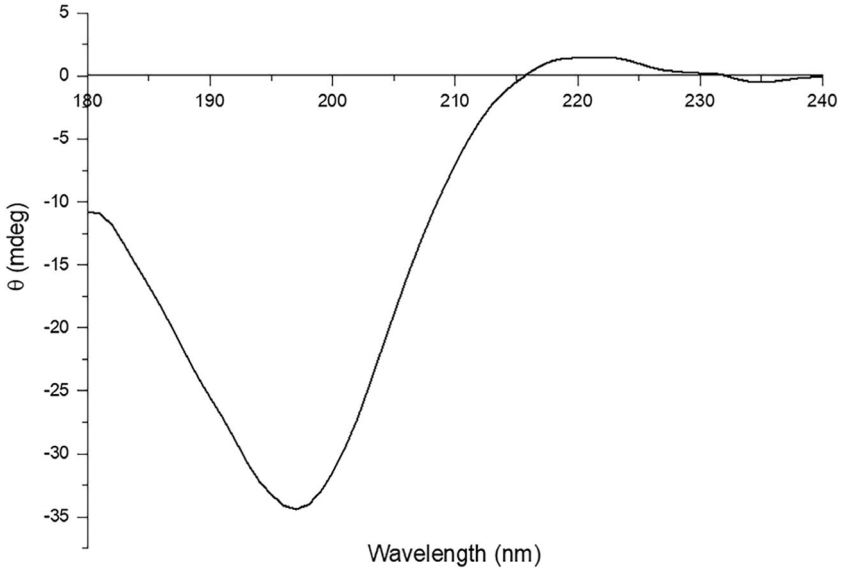

Fig. 4 Circular dichroism spectra of AWC from cod skin measured at 4 °C

third residue throughout most of the collagen molecules, with few exceptions [22].

Proline and hydroxyproline are important for the structural integrity of collagen, with the latter being barely specific of collagenous proteins and used as identification market for this class of proteins. The total proline contents of AWC was 152 residues/1000 residues, with a hydroxylation degree of 38%. This is consistent with other results found in literature, namely the ones obtained with ocellate puffer fish [23], African catfish and salmon [24], and Nile tilapia [25]. More remarkably, these results are equivalent to the ones found in ASC, which supports the effectiveness of the established procedure for the production of collagen from fish skins. Additionally, it is also compatible with the association of total proline contents and hydroxylation degree with the temperature of the living environment, which would reflect a development adaptation, where smaller values are observed for fish species, when compared with mammals, given the aquatic and colder environment of the former group [22].

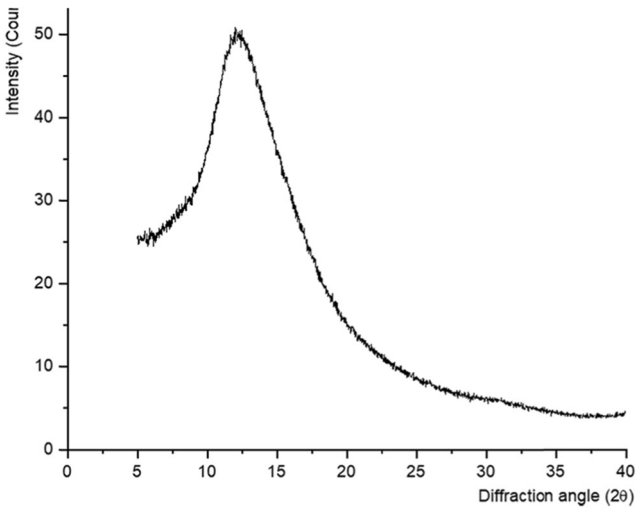

Fig. 5 X-ray diffraction spectra of AWC from cod skin

Micro-DSC measurements

Micro-DSC was used to investigate the process of heat-induced denaturation of collagen. According to the obtained results, AWC exhibited an endothermic peak at 32.3 °C, taken as denaturation temperature (Td), corresponding to the disruption of hydrogen bonds between neighbour peptide chains and the consecutive disorganization of the triple helix structure. Żelechowska et al. [7] and Tylingo et al. [24] reported lower values for the denaturation temperature of Baltic cod collagen (14.4 °C and 15.2 °C, respectively). Although differences on the methodology used for the determination of denaturation temperature may be partially responsible for such variation, the Td determined for AWC is higher than the one detected for ASC (20.9 °C), using the same methodology. This result may be associated with the extraction process that possibly confers a stabilization of the collagen structure, associated with the high pressure used in this study or the weaker acidity of solubilized CO2 when compared with acetic acid. IN fact, Skierka et al. [26] observed that when using stronger acids, as HCl and citric acid, there was a higher denaturation of collagen than when using weaker acids as acetic acid.

Table 2 Molecular weight and polydispersity index of AWC, determined by size exclusion chromatography: Mn – Number Average Molecular Weight; Mw – Weight Average Molecular Weight (Mw); PDI – polydispersity index, determined as Mw/Mn

Collagen	Mn	Mw	PDI
AWC	102.8 ± 7.1	148.8 ± 4.4	1.445 ± 0.057

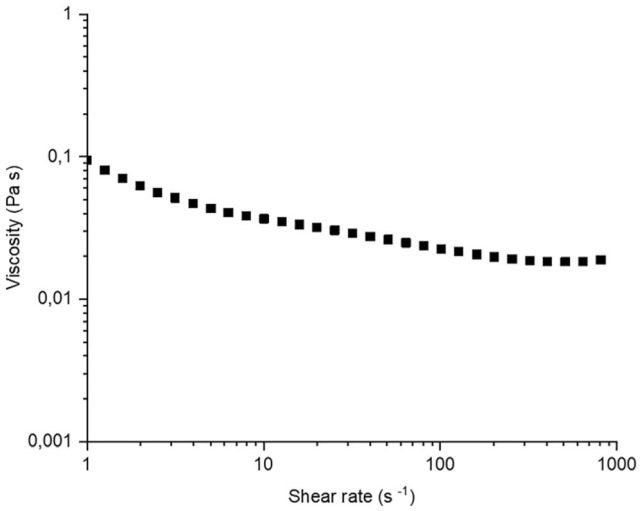

Fig. 6 Viscosity curve of AWC (3 wt%) from cod skin, as a function of shear rate

Circular dichroism analysis

CD was performed to assess the structural organization of the proteins in the extracts, as alpha chains, random coils and triple helices are responsible for very distinct and characteristic signals. In particular, a collagen extract in which the protein keeps its native structure, with preserved triple helix, is characterized by a CD spectrum exhibiting a positive peak at 221 nm (maximum positive cotton effect) and a negative peak at 198 nm (maximum negative cotton effect), with a crossover point (zero rotation) at approximately 213 nm [25, 27]. The CD spectrum of AWC, illustrated in Fig. 4, shows clearly the positive peak at 220 nm, an intense negative peak at 195 nm, with a crossover point at 215 nm, consistent with the presence of intact triple helix in the AWC sample, corroborating the information previously obtained from the FTIR spectrum analysis, and also reported from others authors [5, 6, 20].

X-ray diffraction

XRD was used to address the conformation of collagen, as it will cause the presence of a repeating pattern giving identifiable signal(s) in XRD due to specific X-ray diffraction. As shown in Fig. 5, the XRD diagram disclosed one sharp peak associated with the distance between the molecular chains of the triple helical structure of collagen [25, 28]. The corresponding diffraction angle (2θ) of this peak are around 12 °, from which pattern distances can be calculated by using the Bragg law, with the d value of 7.37 Å. The results demonstrate that AWC is in its native and non-denatured conformation, in agreement with the previous observations from CD and FTIR analysis.

GPC-SEC for molecular weight determination

The determination of the Number Average Molecular Weight (Mn) and Weight Average Molecular Weight (Mw), as well as the polydispersity index (Mw/Mn), was performed by size exclusion chromatography, where molecules with higher hydrodynamic radius will be eluted in shorter time. This hydrodynamic radius can be influenced by the molecular size, as well as by its conformation, being critical to use appropriate standards for calibration and adequate models for molecular weight determination. The obtained result shown that the AWC had a molecular weight of about 149 kDa (Table 2), compatible with the observations made by SDS-PAGE, and the results reported by Silva et al. [17] for marine sponges collagen.

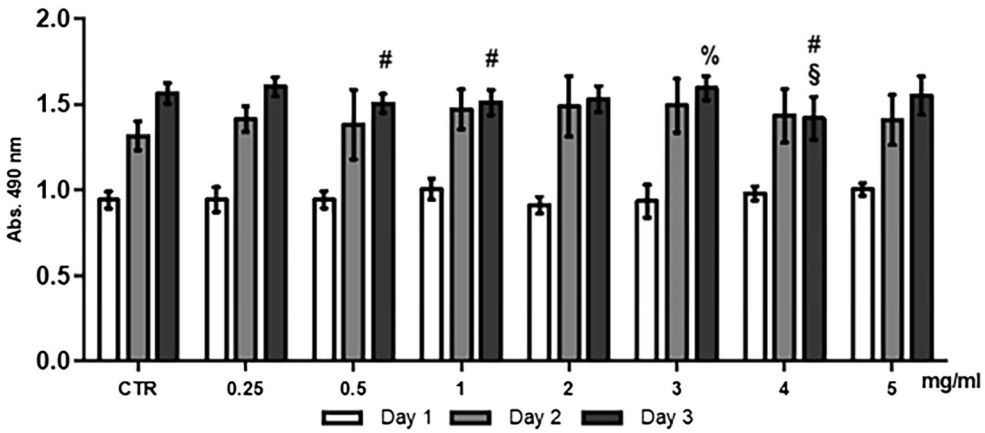

Fig. 7 Metabolic activity, measured as result of MTS assay, of human fibroblast cell line (MRC-5) cultured in the presence of AWC, at different concentrations, during up to 3 days. Data were considered statistically different if $p < 0.05$. * indicates significant differences when compared to CTR; #, when compared to 0.25 mg/mL; %, when compared to 0.5 mg/mL; &, when compared to 1 mg/mL; +, when compared to 2 mg/mL; §, when compared to 3 mg/mL

Rheological properties

The rheological properties of AWC as a function of shear rate were addressed, with the variation in viscosity of AWC solution as a function of shear rate being depicted in the graph of Fig. 6. The extracted collagen have shown a typical shear thinning behavior, characterized by a decrease in the viscosity with increasing shear rate and a linear relationship between the shear rate and shear stress [17]. Moreover, flow behavior was described by fitting of the experimental data (shear stress-shear rate) with the power law model (Eq. (3)): $\tau = K \cdot \dot{\gamma}\eta$, where τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), k is the consistency coefficient (Pa.sn) and η is the flow behavior index [29]. AWC presents a $\eta = 0.73 \pm 0.08$, characteristic of non-Newtonian fluids (for Newtonian liquid, $n = 1$, pseudo plastic fluid $n < 1$ and swelling plastic fluid $n > 1$).

The viscosity behavior is important not only in terms of characterization of the intrinsic properties of the materials, but also towards application development. Like hyaluronic acid and others materials, AWC revealed pseudo plastic features, demonstrating good rheological properties that could be further explored on the synthesis of hydrogels for cosmetic and biomedical applications, namely considering injectable use, should their biological properties and performance be compatible with those aims.

Cytotoxicity

Being collagen a biopolymer of major importance in the biomedical field and given the results previously shown, namely the shear thinning behavior, the development of injectable hydrogels is clearly suggested. Although not being the goal of the present work to develop such biomaterials, it is important to assess the eventual cytotoxicity of the extracted collagen, which would hamper any application in the biomedical sector as would mean that toxic compounds were also present in the produced samples. The influence of the presence of AWC at different concentrations over the viability of MRC-5 human fibroblasts was measured during up to 3 days, as assessment of eventual cytotoxicity. The results from MTS test, measuring the cell metabolic activity, are illustrated in Fig. 7, showing that the extract produced no cytotoxicity when in concentrations up to 5 mg/mL, suggesting an in vitro safety supporting further developments in biomaterials science and corroborating its application for use in the biomedical field.

Conclusions

The supercritical fluids technology using dense CO_2 to acidify water is a potential procedure for collagen isolation and was employed to produce AWC from Atlantic cod skins, with an increased yield in respect to the conventional methodology based in acetic acid solution at atmospheric pressure. The SDS-PAGE profile is consistent with type I collagen and together with amino acid analysis have shown the efficacy of the extraction methodology, given the similarity with collagen obtained using the conventional methodology. According to the results from FTIR, XRD and CD analyses, AWC maintained its native and intact triple helix structure upon extraction, which might be relevant regarding the expression of some biochemical and biological properties dependent on protein structure. Moreover, the obtained extract presented interesting rheological performance, with shear thinning behavior, which together with the non-cytotoxic character, suggests their use on the development of cosmetic formulations or as injectable biomaterials for application in biomedical context.

It is noteworthy that this is an environmentally sustainable process, not only because represents a biotechnological strategy to valorize a fish by-product but also because the methodology itself carries a reduced consumption of water and time when compared with the conventional method, keeping the possibility to be scalable to an industrial plant. The obtained higher yield would support the demand resulting from their use in the biomedical, cosmetic, and pharmaceutical areas, with clear economic benefits and will be the subject of further studies.

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Compliance with ethical standards

Conflict of interest The authors declare no competing financial interest.

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