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# Valorization of Arapaima *gigas* processing residues as a sustainable source of collagen, gelatin, chitin and chitosan

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# Abstract

*Arapaima gigas* is an important source of protein in the diet of the Amazonian population, and the skin and scales, by-products of its processing, are inadequately disposed of, thereby affecting the ecosystem. Such by-products are also rich in proteins and polysaccharides, including collagen, gelatin, chitin, and chitosan, which are widely used across industries for their functional properties and accessibility. This study aimed to assess the feasibility of recovering collagen, gelatin, chitin, and chitosan from Arapaima gigas processing residues. Centesimal and mineral composition analyses, together with Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM), were used to characterize the recovered biomolecules and demonstrate their potential for biotechnological applications within a sustainable bioeconomy. This research used pirarucu skin and scales and showed that these residues are a potential source of industrially relevant biomolecules, such as collagen, gelatin, chitin, and chitosan, highlighting their potential as sustainable raw materials for high-value biomaterials and contributing to the development of the Amazonian circular bioeconomy.


# Introduction

The search for new opportunities to generate income and strengthen the bioeconomy by using local renewable resources, such as biomolecules extracted from fishery residues to supply biotechnological products, underpins the implementation of a green economy supply network [1–5]. The fishery production chain generates large amounts of waste, with estimates ranging from 70% to 90% of processed fish, depending on species, size and weight, and the type of processing used [6–8].

In larger species, such as *Arapaima gigas* (Fig. 1), which is known as “pirarucu” in Brazil and “paiche” in Peru, residues represent about 56% of the total weight of the fish, making it extremely important to make use of them, especially to obtain diverse biomolecules such as collagen [9, 10]. *Arapaima gigas* is an important protein source in the Amazonian diet, and its residues are equally rich in protein; however, these residues are often disposed of improperly in rivers, which can lead to ecosystem degradation [11].

Fishery residues are rich in chitin, a linear polysaccharide with some deacetylated monomer units, composed of N-acetylglucosamine residues linked by β-glycosidic bonds and characterized by the presence of nitrogen, which forms long fibers and cannot be digested by vertebrates (Fig. 2) [13, 14]. Chitin has a high molecular weight, is insoluble in water and organic solvents, and serves as a structural component of fungal cell walls and arthropod exoskeletons (insects, lobsters, and crabs). Crustaceans, such as shrimp and lobster, are well known as sources of chitin [15, 16]. In nature, chitin can occur with α, β, and γ crystalline structures, differing by the number of chains per cell, degree of hydration, and unit size [17].

To obtain chitin, strong acid and alkaline solutions are used, such as 50% sodium hydroxide (NaOH) at 100°C, which breaks chitin polymer chains and converts it into chitosan, a deacetylated form of chitin. Chitosan, in turn, is soluble in water and acidic solutions because of the free amino groups in its structure, with a degree of deacetylation of 50% [19, 20].

Chitosan is a versatile biomolecule used for various purposes because of its good mechanical strength, permeability, and solubility in aqueous and acidic solutions. Among its industrial applications, its use as bioparticles, films, sponges, membranes, gels, and fibers [21], edible antimicrobial coating [1, 22], a therapeutic agent [3], in the production of food packaging and coatings with antioxidant and antimicrobial capacity [5, 23, 24], and as a coagulating agent in water for human consumption [25] can all be highlighted. However, when used as an aid in reducing the absorption of fat and cholesterol, its consumption should be associated with a balanced diet and healthy lifestyle habits [26]

The interest in obtaining chitin and chitosan from fishery residues stems from the potential to generate new sources of bioeconomic income while reducing environmental pollution. Currently, most of the industrially obtained production of chitin is from shrimp, crab, and lobster shells [19], and these residues have chitin (20–30%), protein fraction (30–40%), inorganic salts (30–50%), mainly calcium carbonate and phosphate, and lipids (0–14%). However, fish scales also provide chitin and chitosan [27].

Beyond the environmental and economic appeal of obtaining biomolecules from fishery residues, demand is also driven by religious beliefs and the prevention of zoonoses and allergies, which also motivate choices about consuming products (aquatic organisms, such as fish) and services [28–30]. These beliefs are defended mainly by Judaism, Islam, Hinduism, and some Christian denominations [31, 32].

The chemical composition of every aquatic organism is directly related to the species, environmental factors, time of year, quantity and quality of nutritional intake, stage of sexual maturity, age, and body fraction [33]. Species such as pirarucu exhibit optimal centesimal and mineral composition compared with other fish species, such as tilapia, making it important to fully exploit pirarucu processing, which is cultivated mainly in sustainable development reserves [10, 11, 34–39].

In this work, we analyzed the proximate and mineral compositions and used FTIR and SEM to assess the quality of alternative biomolecule sources, primarily derived from fishery residues.

## Materials and methods

Fazenda Tony, located at KM 33.200 on the Iquitos-Nauta highway, Peru (-4.032044514666043, -73.4338011532962), donated the samples used in the study. We used a 2 kg sample of pirarucu scales and skin collected in October 2021. After cleaning, we packaged, froze, and transported the samples to the Food Engineering Laboratory (FIIA/UNAP) in Iquitos, Peru. After analyzing the extracts in January 2022, we packed the obtained material and the raw material and transported them to Manaus, to the Fish Technology Laboratory (FCA/UFAM), for proper storage and further analysis at the Enzymology Laboratory (Biosciences Center - UFPE) in Recife, Brazil.

The scales were dried in an oven at 34°C for 12 h. After this step, they were ground, packed in bags, and placed in a desiccator to await the subsequent analyses. The skin was cut into small pieces, placed in bags, and stored in the cold until further use.

# Centesimal composition

# Moisture

Moisture was determined by drying the samples in an oven at 105°C until they reached a constant mass, according to the Association of Official Analytical Chemists method [40].

## Ash

The 1.5 g sample was placed in a porcelain crucible and incinerated in a muffle furnace at 550 ºC for 8 hours. The sample was removed from the muffle furnace, transferred to the desiccator, and weighed. The calculations were performed in accordance with the standards [40], with values expressed as % ash.

## Crude protein

Crude protein was determined using the Kjeldahl method, in which total organic nitrogen content was quantified according to the AOAC method [40]. For digestion, 0.02 g of the sample was weighed on paper to prevent adhesion to the tube walls, then 5 mL of sulfuric acid ($H_2SO_4$) and 1.0 g of catalytic mixture were added. The tubes were then placed in the digester block at 350°C for approximately 3 hours, until complete digestion, indicated by a translucent green color. After digestion, the samples were distilled using a nitrogen distiller. 25 mL of 40% sodium hydroxide (NaOH) was added to a 1 M boric acid ($H_3BO_3$) solution to a total volume of 5 mL. The solution was then distilled, after which the nitrogen in the sample was determined by titration with 0.1 N hydrochloric acid (HCl). The conversion factor used was 6.5/1.14 [41]. Results were expressed as % crude protein.

## Total lipids

Total lipids were determined by the Soxhlet method, according to the AOAC method [40].

# Carbohydrates

Carbohydrates were determined as the sum of the percentages of moisture, protein, lipid, and ash, as described in the AOAC method [40].

## ∑= 100 – (A + B+C + D) (1)

Where A = total protein, B = ether extract (lipids), C = moisture, D = ash

## Fiber

Total fiber was determined using the fiber determiner (TE 149 TECNAL®) with 0,5 grams of dried, deglazed sample, sulfuric acid 0,225 M, and sodium hydroxide 0,313 M for acid and alkaline hydrolysis, respectively [42].

# Gelatin extraction

We used Monte's method [43], with some modifications. We sanitized the scales, dried them at 40°C for 24 h, and then ground them. Using 100 g of scales, the alkaline extraction process was started in 0,1 M NaOH solution (1:10 w/v) for 24 h, followed by the demineralization process with 10,1 M at 60°C for 3 h, and the second in 0,1 M $CH_3COOH$ solution at 25°C for 3 h, which left the scales ready for the gelatin extraction step. We performed this extraction with deionized water at 45°C for 24 h. After 24 h, the liquid phase (gelatin) was separated, then underwent precipitation and purification with 2,6 M NaCl, addition of HCl P.A., dialysis, and lyophilization, and was named pirarucu scale gelatin (PScG). We used the solid fraction, consisting of scale residues remaining after the described process, for chitin and chitosan extraction.

# Chitin and chitosan extraction

We extracted chitin and chitosan according to Cahú et al. [19], with some modifications. We acid-extracted the solid residue from the previous step with 0.1 M HCl at 25°C, then alkaline-extracted it with 1 M NaOH at 70°C for 1 h to obtain chitin (PCh). To obtain chitosan (PCht), deacetylated chitin in 50% (w/v) NaOH solution at 100°C for 24 h. The degree of deacetylation was determined according to the formula:

$$\%DD = 100 - \left[\frac{A1655}{A3450} * \frac{100}{1{,}33}\right]$$

2

$A_{1655}$= absorbance at a wavelength of 1,655 $cm^{-1}$, $A_{3450}$ = absorbance at a wavelength of 3,450 $cm^{-1}$.

Figure 3 shows a modified procedure for obtaining gelatin, chitin, and chitosan, as described by Cahú and Monté [19, 43].

# Collagen extraction

We performed collagen extraction as described by Carpio [10], with some modifications. This process began with alkaline extraction using a 0.5 M NaOH solution at a 1:20 (w/v) ratio, added to the fractionated skins without meat residues for 12 h. Next, extract the material with 10% (w/v) butyl alcohol ($C_4H_9OH$) for 12 h. Perform all steps in an ultra-thermostatic bath set at 20°C. Finally, perform an acid extraction in 0.5 M $CH_3COOH$ solution (1:40 w/v) for 24 h. Precipitate the extracted collagen by differential saline separation through successive addition of 0.9 M sodium chloride (NaCl) solution to a 0.9 M sodium carbonate-bicarbonate buffer solution (pH 10), followed by centrifugation at 10000 rpm

for 40 min at 4°C. The resulting solution was dialyzed, lyophilized, and designated acid-soluble collagen (ASC). The process yield was then calculated. Figure 4 shows the schematic of the collagen-obtaining process.

FTIR was performed on a Fourier-transform infrared spectrophotometer (Shimadzu, IRTracer–100), averaging 16 scans over the spectral range 4000–400 cm, using attenuated total reflectance (ATR) with a zinc selenide (ZnSe) crystal. The samples were measured by pressing them against a prism approximately 1,8 mm in diameter, positioned horizontally.

Scanning electron microscope (SEM) images were obtained using a Vega 3 (Tescan) coupled to an energy-dispersive X-ray spectroscopy (EDS) system (INCA energy, United Kingdom).

## Result and discussion

# Centesimal and mineral composition

Table 1 presents the proximate composition percentages of pirarucu skin and scales, including moisture, fibers, and lipids.

Table 1
Centesimal composition of pirarucu skin and scales

| Skin | Moisture (%) | Ash (%) | Proteins (%) | Lipids (%) | Carbohydrates (%) | Fiber (%) |
|---|---|---|---|---|---|---|
| | **67.75** | **0.05** | **23.01** | **7.35** | **1.84** | **-** |
| Scales | 12.47 | 34.46 | 43.49 | 0.76 | 8.82 | 5.54 |

Table 2 shows the mineral composition percentages in pirarucu scales.

Table 2
Mineral composition of pirarucu scales

| Element | % |
|---|---|
| C | 54.84 |
| O | 35.94 |
| Al | 0.20 |
| P | 2.06 |
| Ca | 5.92 |
| Nb | 0.72 |
| Pt | 0.00 |
| Na | 0.23 |
| Cl | 0.08 |
| Total | 100.00 |

# Extraction yield of recovered biomolecules

Table 3 presents the extraction yields of biomolecules recovered from *Arapaima gigas* processing residues. Acid-soluble collagen (ASC), gelatin (PScG), chitin (PCh), and chitosan (PCht) were successfully obtained from pirarucu skin and scales, demonstrating the feasibility of an integrated valorization strategy for fish-processing by-products. Although extraction yields varied among the biomolecules, all recovered materials retained structural integrity, as confirmed by FTIR and SEM analyses, indicating their suitability for subsequent biotechnological applications.

Table 3
Extraction yields of biomolecules recovered from Arapaima gigas processing residues.

| Biomolecule | Raw material | Yield (%) |
|---|---|---|
| Acid-soluble collagen (ASC) | Skin | 2.29 ± 0.69 |
| Gelatin (PScG) | Scales | 4.03 ± 0.33 |
| Chitin (PCh) | Scales | 7.47 ± 0.74 |
| Chitosan (PCht) | Scales | 1.34 ± 0.47 |

The acid-soluble collagen yield obtained from pirarucu skin was 2.29 ± 0.69%, which is considerably lower than the values previously reported for the same species (24.6–27.7%). This lower recovery may reflect differences in extraction conditions, raw material characteristics, collagen cross-linking, or purification procedures. Despite the reduced extraction yield, FTIR and SEM analyses confirmed the

preservation of the characteristic collagen structure, suggesting that extraction efficiency may be further optimized without compromising molecular integrity.

The gelatin yield reached 4.03 ± 0.33%, which is within the lower range reported for fish scales from other species, such as tilapia (approximately 5–16%). This result demonstrates that pirarucu scales constitute a promising alternative source of fish gelatin, although further optimization of extraction temperature, extraction time, and pretreatment conditions may improve recovery.

The chitin yield obtained from pirarucu scales was 7.47 ± 0.74%, slightly lower than values commonly reported for other fish species (approximately 10–20%). Considering that fish scales have lower chitin contents than crustacean shells, the obtained yield confirms the feasibility of using Arapaima gigas scales as an alternative source of this polysaccharide.

Following deacetylation, we obtained a chitosan yield of 1.34 ± 0.47%. Although this value is lower than those reported for several commercial fish species, the FTIR spectrum confirmed successful deacetylation with a degree of deacetylation of 63%, demonstrating that the extracted material possesses the characteristic chemical structure of chitosan. Optimization of the deacetylation process may further improve yield while maintaining polymer quality.

# Spectroscopy characterization

Figure 5 shows the FTIR spectra of Pirarucu. Figure 5a shows the acid-soluble collagen of the skin, Fig. 5b shows the gelatin of the scales, Fig. 5c shows the chitin of the scales (PCh), and Fig. 5d shows the chitosan of the scales (PCht). The spectra in Fig. 5 show main functional-group bands at 3281, 3292, 3066, 2918, 1651, 1636, 1634, 1630, 1539, 1537, 1558, 1568, and 1235 $cm^{-1}$.

# Morphological characterization

The SEM images of Pirarucu shown in Fig. 6 for Pirarucu acid-soluble collagen in skin (ASC) (see Fig. 6a), scale gelatin (PScG) (see Fig. 6b), scale chitin (PCh) (see Fig. 6c), and scale chitosan (PCht) (see Fig. 6d) were obtained at different scales.

## Discussion

This study analyzes protein biomolecules (collagen and gelatin) and polysaccharides (chitin and chitosan). By examining and comparing undecimal (percentage-based) and mineral composition data, we can determine whether pirarucu skin and scales serve as potential sources of the biomolecules investigated. This approach allows for a clearer understanding of the nutritional and functional properties inherent in these aquatic resources.

The centesimal composition data for pirarucu skin (Table 1) are like those reported in previous studies, both for the skin [10] and for fillets of the same species [11, 35]. The protein content of 23.01% in the skin and 43.49% in scales (Table 1) shows that it is a potential source of protein biomolecules and can

be compared with other sources of biomolecules, such as tilapia [39], which is one of the most reported aquatic species for collagen and gelatin extraction. It was also observed that the protein content in the pirarucu scales is even higher than in the skin, since the scales have two layers: one highly mineralized on the outside and the other compensated by collagen fibrils on the inside (Bouligand) [44]. This makes scales a better source of collagen and gelatin than skin.

Chitin and chitosan are derived from the long-chain N-acetylglucosamine derivative, and these biomolecules also belong to the carbohydrate group [45]. The carbohydrate content (Table 1) in pirarucu scales (8.82%) is higher than that in tilapia scales (4.76%), as reported by other authors [38]. Because chitin and chitosan are part of the scales' bone structure, it was important to evaluate ash content, which also reflects the scales' mineral content. Ash values in pirarucu scales (34.46%) are like those in tilapia scales (19.72% and 40.0%) reported by other authors [38, 39].

The mineral content of the scales (Table 2) indicates that Ca is the predominant mineral in pirarucu scales, as reported by other authors [34]. This mineral content makes demineralization, deacetylation, and purification critical steps [13, 46, 47] for obtaining high-quality biomolecules for biotechnological applications.

FTIR has been widely used to identify proteins and polysaccharides by analyzing the functional groups present in the molecules and determining their characteristic molecular signatures. As a result, the technique is widely used to characterize biomolecules of industrial interest, such as collagen, gelatin, chitin, and chitosan, and to assess their integrity and purity [19, 48–50].

Figure 5 shows the FTIR spectrum of pirarucu. Characteristic peaks of each biomolecule appear within ranges corresponding to their functional groups. Authors [50] and [51] discuss the characteristics of type 1 collagen. The spectra obtained from the ASC (Fig. 5a) and PScG (Fig. 5b) were like those reported by other authors [10, 38, 52], and the amide A and B bands and the amine I, II, and III bands were identified. According to Cahú et al. [19], the chitosan fingerprint is increased band intensity in the 1590 $cm^{-1}$ range and a proportional decrease in the 1655 $cm^{-1}$ range compared to chitin. These changes indicate that chitin was deacetylated to obtain chitosan, as evidenced by the degree of deacetylation. In the PCh spectrum (Fig. 5c), in the region indicated in the graph, an intense band is observed at 1630 $cm^{-1}$, which corresponds to the stretching vibration of amide group I (C = O) and the superimposed CaO group linked to the OH group via an H-bond. The band at 1537 $cm^{-1}$ is due to N-H deformation of the amide group II. Several authors [18, 27, 53] report a characteristic chitin peak in the 1626 $cm^{-1}$ range, which falls within the 1800 – 1390 $cm^{-1}$ range associated with stretching vibrations. In the PCht spectrum (Fig. 5d), characteristic bands are observed in the indicated region at 1651 $cm^{-1}$ and 1568 $cm^{-1}$, indicating chitin deacetylation (N-H deformation of amines) with a degree of deacetylation of 63%. The bands that appear at lower values indicate the presence of C-O bonds and glycosidic bonds, C–O–C, characteristic of the composition of the polysaccharide. The intensity of the bands between 1700 and 1600 $cm^{-1}$ shows the molecule's characteristic fingerprint.

Scanning electron microscopy (SEM) is an important electron microscopy technique that provides high-quality, detailed images of particles with high spatial resolution and is a useful tool for material characterization [54]. Therefore, SEM is important for obtaining characteristic images of the biomolecules under study [54–56].

In the images obtained using SEM (Fig. 6a-d), it is possible to observe the ASC, PScG, PCh, and PCht biomolecules obtained from the skin and scales of the pirarucu. In Fig. 6a, fine collagen fibers are visible, connected to each other, but rods also appear that, according to some authors, indicate the presence of inorganic materials, such as carbonate [56, 57]. The presence of this compound may be due to incomplete purification, for example, when the collagen was extracted with $CH_3COOH$, a sodium carbonate-bicarbonate buffer solution was required. Therefore, we recommend a full dialysis step to obtain a fully purified biomolecule. The PScG in Fig. 6b resembles images reported by other authors [54]. The PCh shown in Fig. 6c is also like that reported by other authors [56, 57], and in Fig. 6d, it is possible to observe the porosity, fibrosis, and irregular rounded shape of the PCht, as reported by other authors [53, 58].

## Conclusions

The analysis confirms that residues from pirarucu processing, i.e., skin and scales, are a potential source of biomolecules. Since the pirarucu is a species of enormous size, a greater number of residues can be obtained during its processing, even more so since it is a species that is cultivated in fish farms and in sustainable development reserves, making its production important in terms of sustainability, not only in local trade, but also in international trade. The sustainable use of residues from pirarucu processing will transform the lives of everyone involved in this production chain. In addition, it will stimulate the bioeconomy, new income streams, and new bioproducts from the biomolecules obtained, such as collagen, gelatin, chitin, and chitosan. Although the extraction yields obtained for collagen, gelatin, chitin, and chitosan indicate that further optimization of the extraction process is desirable, the successful recovery and physicochemical characterization of these biomolecules demonstrate the feasibility of valorizing Arapaima gigas processing residues as a renewable feedstock for high-value biomaterials within an integrated Amazonian biorefinery framework. These findings support implementing integrated biorefinery strategies for Amazonian fish processing residues, contributing to sustainable resource utilization and the development of high-value biomaterials.

## Declarations

# Author Contribution

K.C.R.C.: Conceptualization, methodology, investigation, formal analysis, Data curation, writing original draft. C.C.G.: Investigation, methodology, formal analysis. C.F.C.F.: Investigation, resources. A.A.M.R.: Formal analysis, writing, review and editing. Y.R.B.: Formal analysis, writing, review and editing. A.G.: Formal analysis, writing, review and editing. R.D.S.S.: Investigation, data curation. T.B.C.: Methodology,

supervision, writing, review, and editing. A.J.I.: Resources, Supervision. W.R.B.: Conceptualization, funding acquisition, writing, review, and editing. R.P.C.: Conceptualization, supervision, resources, writing, review, and editing. All authors reviewed the manuscript.

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# Figures



**Figure 1**

Illustrative figure of the Pirarucu (*Arapaima gigas*) [12].

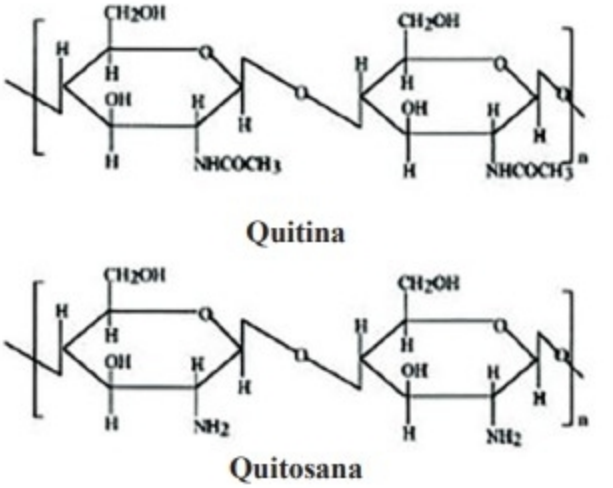



**Figure 2**

Chemical structure of chitin and chitosan [18].

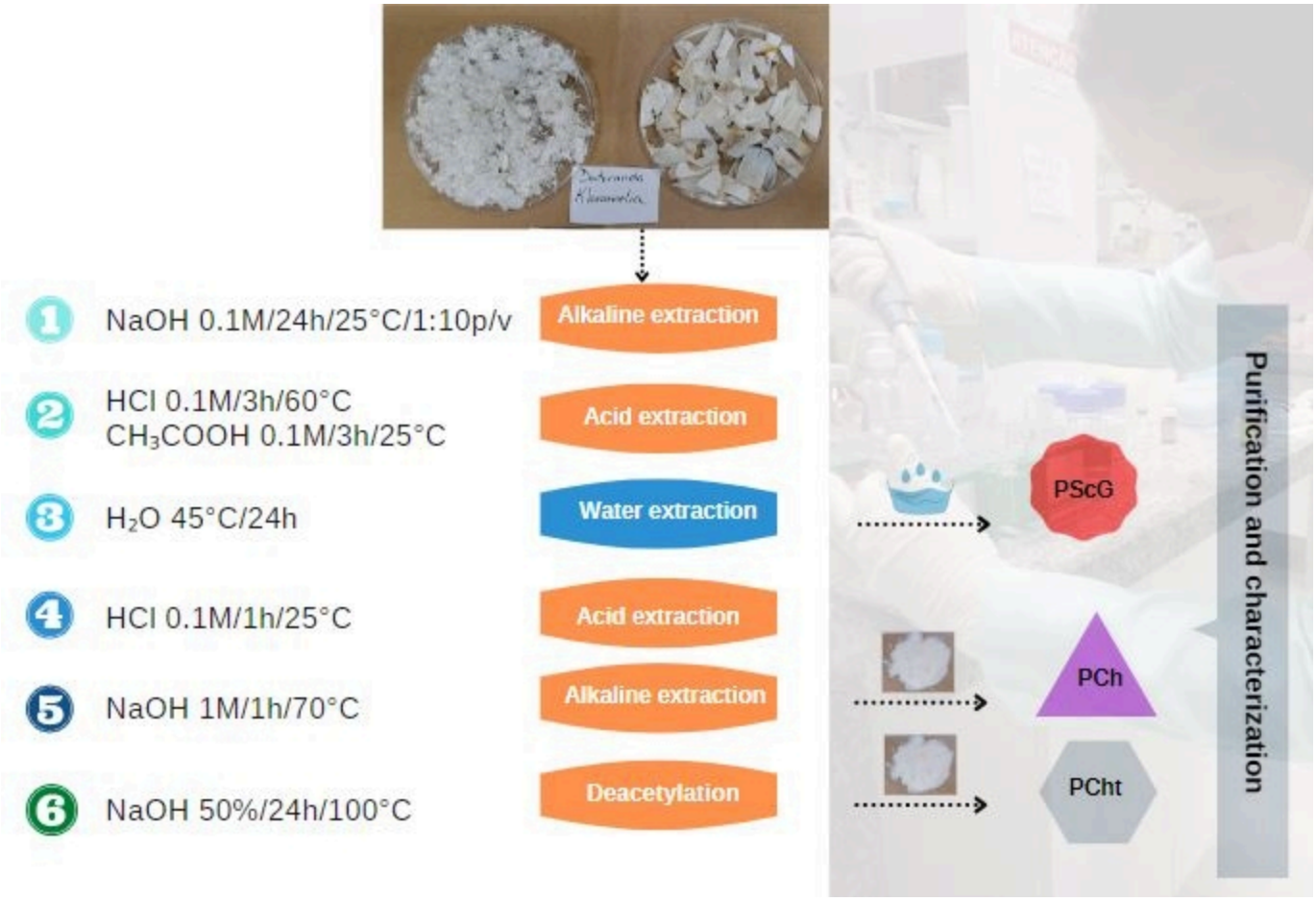



**Figure 3**

Scheme of the procedure for obtaining gelatin, chitin, and chitosan.

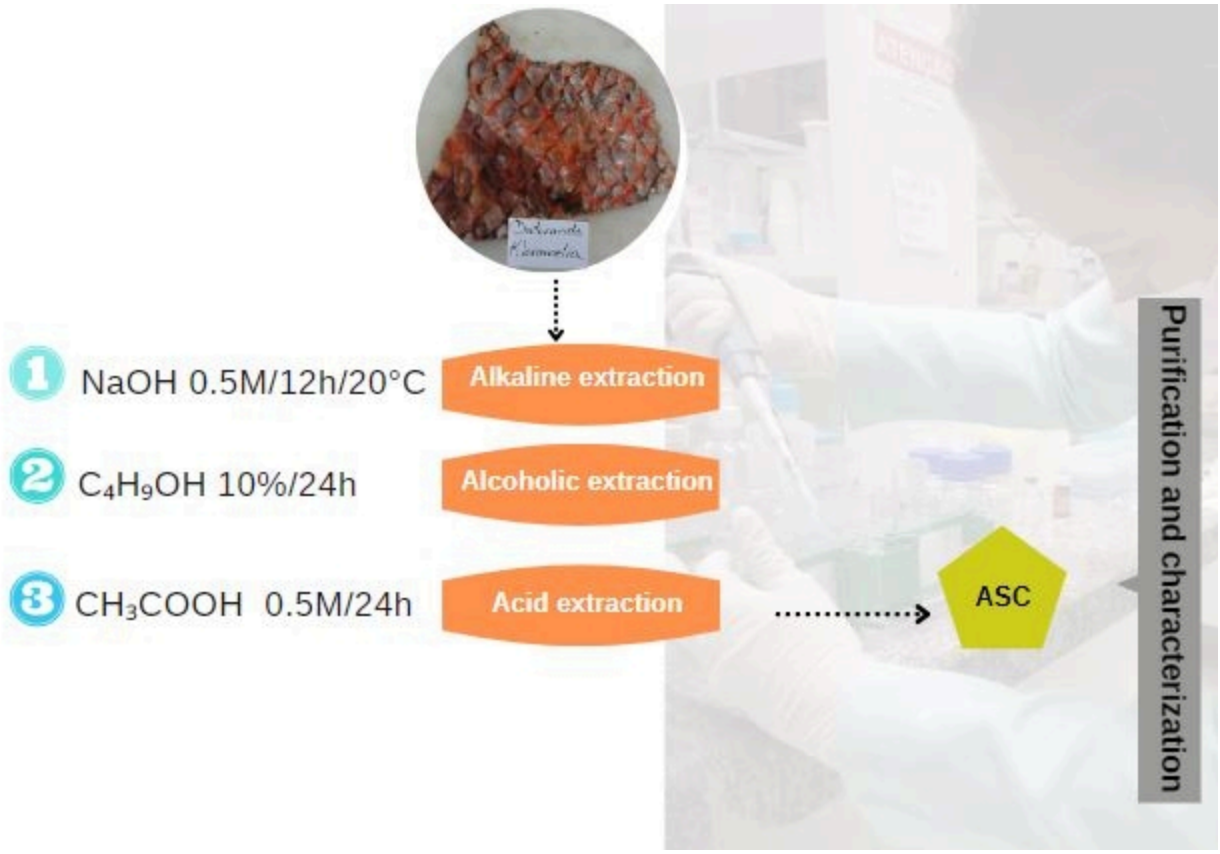



**Figure 4**

Scheme of the procedure for obtaining collagen.

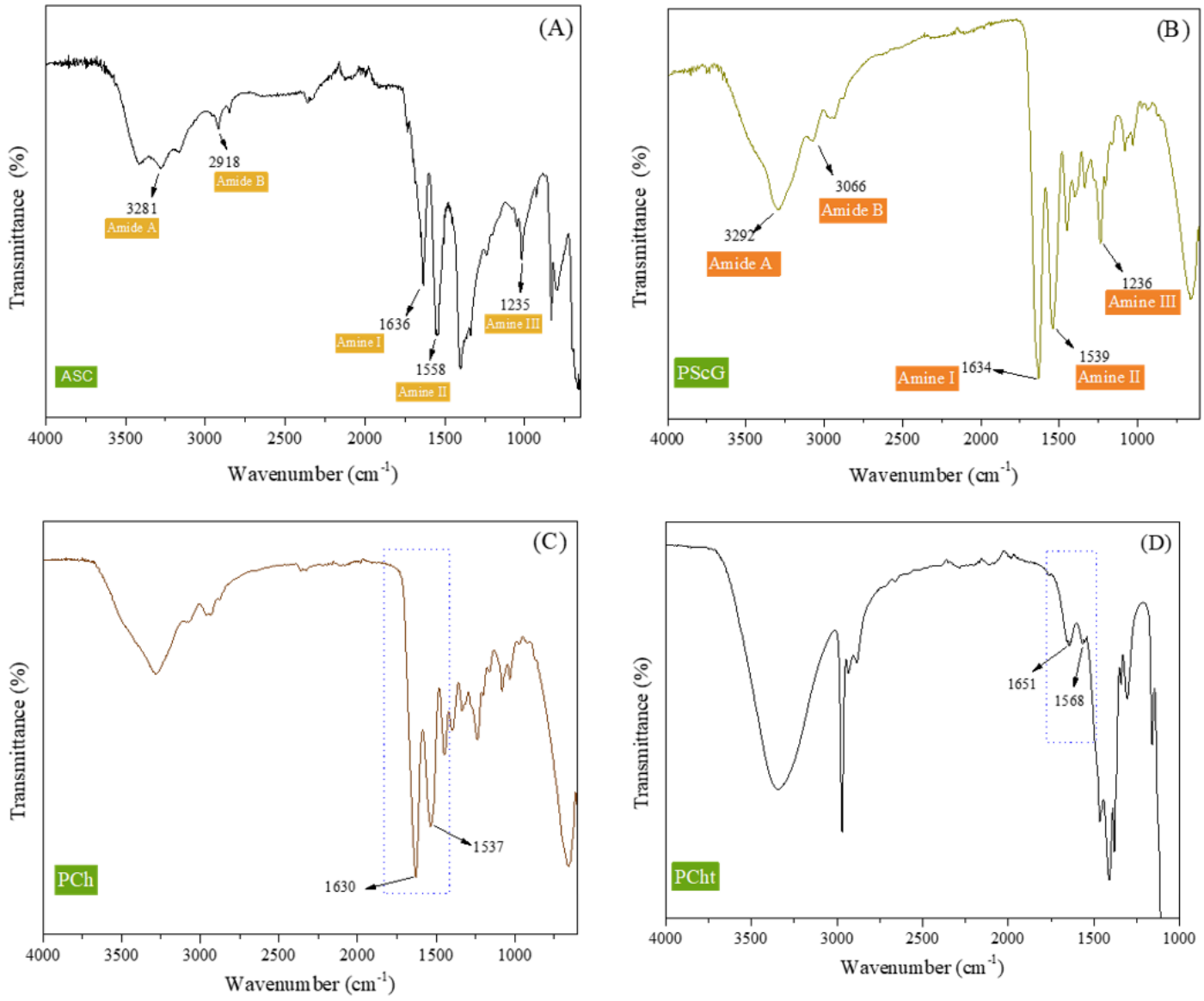



**Figure 5**

FTIR spectrum of (A) acid-soluble collagen from pirarucu skin (ASC), (B) gelatin from pirarucu scales (PScG), (C) chitin from pirarucu scales (PCh), and (D) chitosan from pirarucu scales (PCht), showing a degree of deacetylation of %DD = 63

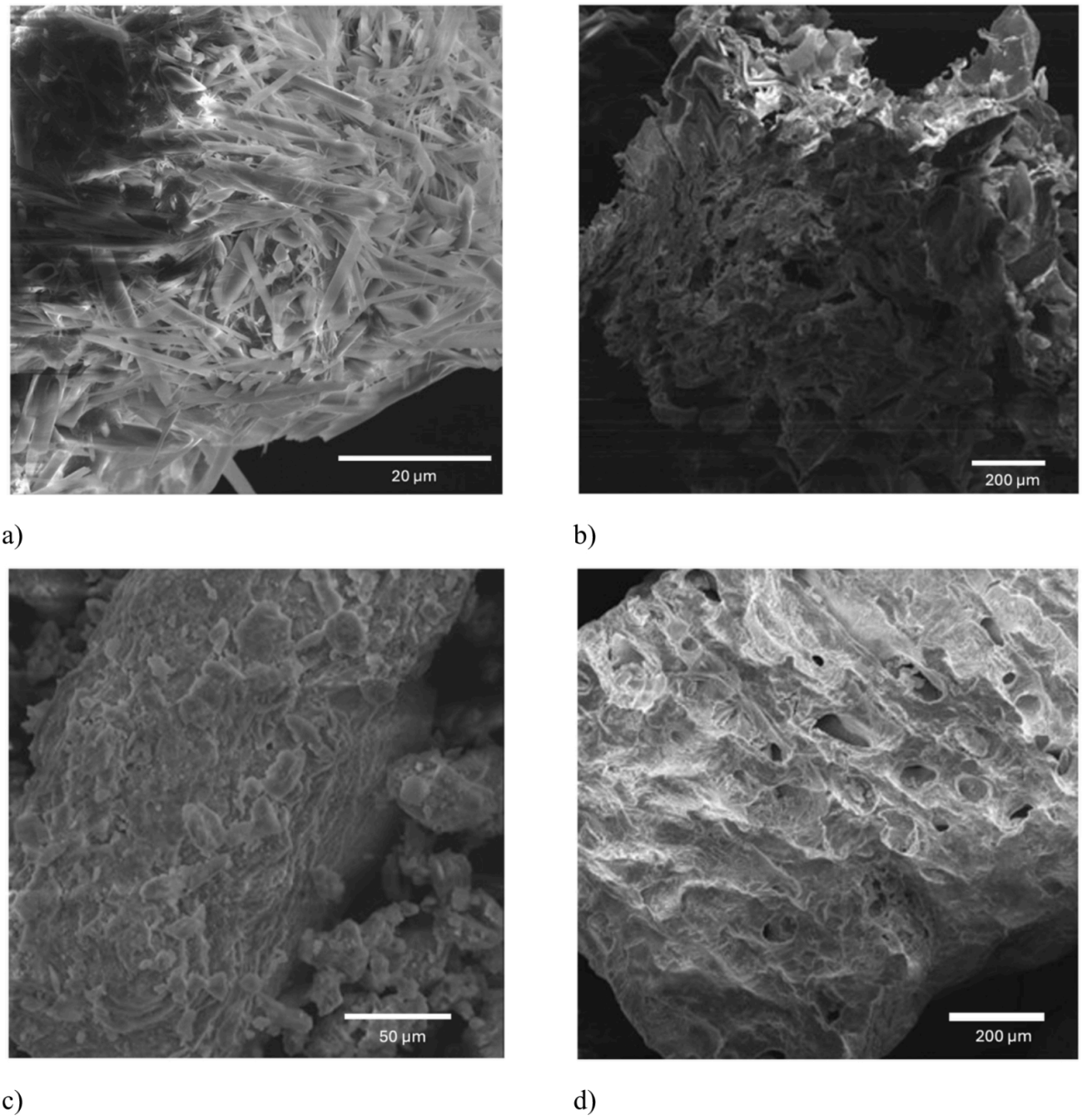



**Figure 6**

SEM image of pirarucu for (a) acid-soluble collagen from skin at 20 µm scale, (b) gelatin scales at 200 µm scale, (c) chitin scales at 50 µm scale, and (d) chitosan scales at 200 µm scale.