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Research Article

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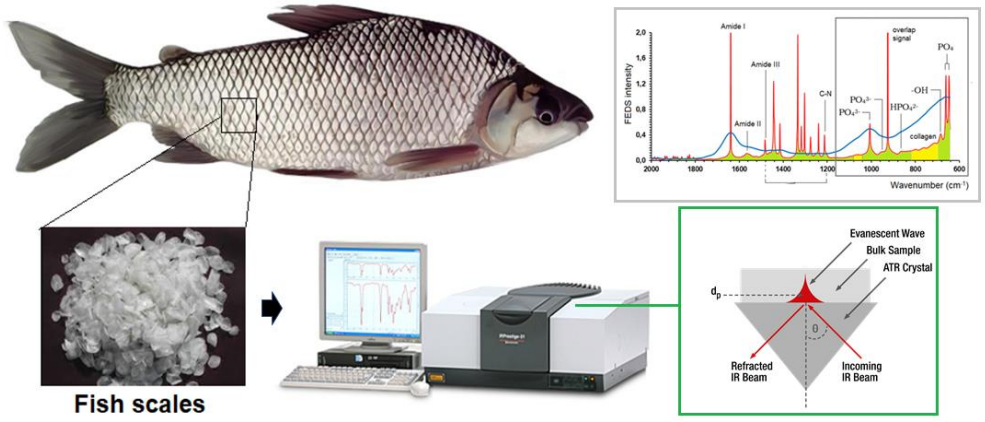
Graphical abstract



Mid-infrared spectral characterization of fish scales: "Bocachico" (*Prochilodus magdalenae*) by functionally-enhanced derivative spectroscopy (FEDS) - A methodological approach

Abstract

Analysis of information obtained from fish scales is one of the most important tools available to biologists, because fish scales capture information on the biotic and abiotic factors influencing populations. Compositional information of surface of fish scale can be obtained by infrared spectroscopy but overlap problem limits the recording of analytical information from spectra. Here, it is proposed the used of functionally-enhanced derivative spectroscopy in order to improve the analysis by infrared spectroscopy of fish scales and advance in the design of analytical methodology to study of fish scale and its use as bioanalytical markers into productive and natural systems. For that, "Bocachico"

Keywords
Fish scale
Prochilodus magdalenae
Bocachico
Functional transformation
Derivative spectroscopy
FTIR

(*Prochilodus magdalenae*) was used as freshwater fish model and mid infrared spectra of scales were obtained by Attenuated Total Reflectance technique. Repeatability and reproducibility were evaluated by Pearson correlation coefficient using correlation matrix methodology. It is concluded that FTIR spectroscopy in conjunction with FEDS transform is a promissory technique for the analysis of biological surfaces of interest for aquiculture science and technology.

Caracterización espectral del infrarrojo medio de escamas de pescado: "Bocachico" (*Prochilodus magdalenae*) mediante Espectroscopia Derivativa Mejorada Funcionalmente (EDMF) - Un enfoque metodológico

Resumen

El análisis de la información obtenida de las escamas de peces es una de las herramientas más importantes disponibles para los biólogos, porque las escamas de peces capturan información sobre los factores bióticos y abióticos que influyen en las poblaciones. La información de la composición de la superficie de las escamas de peces se puede obtener mediante espectroscopía infrarroja, pero el problema de la superposición limita el registro de información analítica de los espectros. Aquí, se propone el uso de espectroscopía derivada mejorada funcionalmente para mejorar el análisis por espectroscopía infrarroja de las escamas de peces y avanzar en el diseño de una metodología analítica para estudiar las escamas de peces y su uso como marcador bioanalíticos en sistemas productivos y naturales. Para eso, se usó "Bocachico" (*Prochilodus magdalenae*) como modelo de pez de agua dulce y se obtuvieron espectros de escamas de infrarrojo medio mediante la técnica de Reflectancia Total Atenuada. La repetibilidad y la reproducibilidad se evaluaron mediante el coeficiente de correlación de Pearson utilizando la metodología de la matriz de correlación. Se concluye que la espectroscopía FTIR junto con la transformación FEDS es una técnica promisoria para el análisis de superficies biológicas de interés para la ciencia y tecnología acuícola.

Palabras clave

Escama de pez
Prochilodus magdalenae
Bocachico
Transformación funcional
Espectroscopía derivada
Caracterización FTIR

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Mid-infrared spectral characterization of fish scales: "Bocachico" (*Prochilodus magdalenae*) by functionally-enhanced derivative spectroscopy (FEDS) - A methodological approach

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Abstract
Analysis of information obtained from fish scales is one of the most important tools available to biologists, because fish scales capture information on the biotic and abiotic factors influencing populations. Compositional information of surface of fish scale can be obtained by infrared spectroscopy but overlap problem limits the recording of analytical information from spectra. Here, it is proposed the used of functionally-enhanced derivative spectroscopy in order to improve the analysis by infrared spectroscopy of fish scales and advance in the design of analytical methodology to study of fish scale and its use as bioanalytical markers into productive and natural systems. For that, "Bocachico" (*Prochilodus magdalenae*) was used as freshwater fish model and mid infrared spectra of scales were obtained by Attenuated Total Reflectance technique. Repeatability and reproducibility were evaluated by Pearson correlation coefficient using correlation matrix methodology. It is concluded that FTIR spectroscopy in conjunction with FEDS transform is a promissory technique for the analysis of biological surfaces of interest for aquiculture science and technology.

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1. Introduction

Fish scales are small rigid plates growing out of the skin of fishes, thus, the skin of most fishes is covered with these protective scales providing effective camouflage through the use of reflection and coloration, as well as possible hydrodynamic advantages. In general, scales show several size, shape, structure, and extent depending of fish species. At the present, approximately 28,000 living species of fish have been identified, of which about 1000 are cartilaginous fishes (i.e., cartilaginous skeleton), about 108 are jawless fishes and the remaining are bony fish (i.e., bone skeletons) (Torres et al., 2008; Gene et al., 2009; Gil-Duran et al., 2016).

Fish scales are classified in four basic types depending on the fish species: Placoid, cosmoid, ganoid and elasmoid scales (Kardong, 2008). *Placoid scales* are found in cartilaginous fish like sharks and manta rays; *cosmoid scales* are found only on ancient lobe-finned fishes, including some

of the earliest lungfishes (subclass Dipnoi), and in Crossopterygii, including the living coelacanth in a modified form; *ganoid scales* are found in the sturgeons, paddlefishes, gars, bowfin, and bichirs, and *elasmoid scales* are found in most of the bony fish (Kardong, 2008).

Elasmoid scales primarily consist of hydroxyapatite (mineral component), type I collagen (organic component) and water. The collagen fibers are arranged in discrete plies stacked on top of one another, each with different orientation (Ikoma et al., 2003; Gil-Duran et al., 2016). Elasmoid scales are also composed of two primary layers. The external limiting layer is highly mineralized and composed of calcium-deficient apatite or calcium carbonate, depending on the fish. The internal layer (elasmodine) is a composite of minerals and type I collagen fibers organized in unique plies. The plies are arranged in the form of a plywood structure. The diameter of the collagen fibers is around 1 μm, and these are constructed of an assembly of fibrils roughly 100 nm in diameter (Chen et al., 2012; Ikoma et al., 2003; Lin et al., 2011).

In general, the differences in mechanical properties with orientation can be attributed to the collagen plies and degree of reinforcement provided by the mineral crystals. As fish scales are composed of many plies of collagen fibers, their orientation will directly affect the mechanical properties (Gil-Duran et al., 2016).

Analysis of information obtained from fish scales is one of the most important tools available to biologists, because fish scales capture information on the biotic and abiotic factors influencing populations.

Fish scale analysis has been extensively used in scientific studies such as: age and growth, population dynamics, interpretation of past bio-diversity, diet analysis of piscivorous species, ecological integrity of large rivers, stock identi-fication, trace-metal contamination, comparative and phylogenetic studies, rapid isolation of DNA, effect of climate change on populations and taxonomic and evolutionary studies (Taylor, 2012).

Despite of the countless studies on age and growth of fish populations based on the study of scales, it has been recognized that the validity and accuracy of such information is very questioned; but also, although ageing of fish scales is a widely described technique gaps in knowledge and vagaries in interpretation and analysis persist because classic studies were based on single populations and often lacked scientific rigor (Taylor, 2012).

Between techniques for the study of fish scale is the back-calculation based analysis. This mathematical tool is very important in the analysis of the age and growth of fish. Since 1990, attention calls have been made aimed at the care and limitations of the technique, and therefore, the risk of obtaining inaccurate and incorrect conclusions (Francis, 1990). In this way, it has been stated that though the Back-calculation technique is widely used it does not appear to be well understood. Specifically, it is suggested that regression methods are commonly used without to recognize that there are two equally plausible back-calculation hypotheses which can lead to significantly different back-calculated lengths (Francis, 1990). A key assumption of back calculation is "the growth increment of the scale is on average a constant proportion of the growth increment of the fish" and instead of passing through the origin, the line must pass through the point where scale radius equals 0 and the length of the fish is a constant. The common practice of fisheries scientist is to set the value of the constant mathematically rather than from biological data producing potential errors. However, the alternative approach based on the potential use of biologically derived values is not possible because these values are not readily available (Francis 1990; Campana 2001; Taylor, 2012). Although, recent studies have used DNA and stable isotope analyses to assess fish populations, their application is often limited because of high cost per scale sample analyzed; further, these techniques require degradation of the scale sample. Thus, a readily available technique capable of providing further ecological information at low cost and without destroying the scale sample would be preferred (Nielsen and Hansen, 2008; Grey et al., 2009; Taylor, 2012). It has been proposed that geometric morphometric approaches could be one solving to limitations of previously described techniques because an individual's morphological characteristics are dictated by abiotic and biotic factors (Taylor, 2012). However, though geometric morphometric analysis of fish scales has been shown to be a good discriminator of genera using a fixed landmark approach, for freshwater fish scales is often see irregular shapes and, in consequence, it is not possible to identify identical locations on all individuals (Taylor, 2012; Gil-Duran et al., 2016).

Since fish populations are influenced by a number of environmental, physical and biological variables, scales contain important information associated with abiotic and biotic factors influencing an individual fish, which in turn can be aggregated with information from other individuals to understand population responses to such factors. Environmental variables vary over a geographic range and subsequently dictating species distribution and life history traits, typically fish populations are influenced by temperature, flow and climate (Grey et al., 2009; Taylor, 2012; Gil-Duran et al., 2016). Therefore, the advance in the development of strategies for the objective analysis of fish scale is required. In this context, it is clear that the surface-level differentiation of scale is an important starting point for analysis previously indicated, in this work is proposed that the analysis of spectral information could be an adequate technique for the advanced study of fish scale. The starting point is the recognizing of fish scale as a complex biomaterial containing useful surface information depending of technique used. Here, we propose the use of vibrational information obtained from of interaction of infrared light with the matter. Thus, compositional information of surface of fish scale can be obtained using an easy, fast and economic technique. In addition, the equipment is the relatively low-cost; it is a non-destructive and very sensitive technique. The main limitation is the overlapping of signals which is obtained when complex samples are analyzed, however, this problem can be easily solved by different data-transform techniques; in particular, we propose the use of Functional-enhanced derivative spectroscopy (FEDS) as alternative for overlapping (Palencia, 2018; Garcia et al., 2018). It is important to say that previous publications related with this approach have not been published as result of relatively recent development of FEDS analysis.

2. Experimental section

2.1 Samples

'Bocachico' (*Prochilodus magdalenae*) was used as fish model and obtained from local market. This specie was selected because 'bocachico' is a tropical freshwater fish from Colombia. This fish is found in the Atrato, Sinú, Cauca and Magdalena Rivers being used as usual foods in much places of Colombian Caribbean cost.
Several fish scales were obtained from several parts of fish body, washed and dried at 45 °C in over of lamellar flow.

2.2. Obtaining of spectra

Mid infrared spectra of fish scales were obtained using an Infrared equipment Thermo Scientific NICOLET 6700 with Attenuated Total Reflectance technique (ATR) in the PIKE MIRacle accessory and ZnSe crystal. Samples were identified as sample 1, 2, 3 and 4 and analyzed in lamellar shape, without mix, by quadruplicate (samples 1 and 2) and by single-one analysis (samples 3 and 4).

2.3. Data processing

Data were transferred to spreadsheet and analyzed using Microsoft excel software. Several algorithms were used depending of stage of analysis. Thus:
For repeatability and reproducibility, an autoscaling of data were initially performed, for that, maximum absorbance (a_{max}) and minimum absorbance (a_{min}) were determined by the use of following functions:

$$a_{max} = max\ (a_1 : a_n) \tag{1}$$

$$a_{min} = min\ (a_1 : a_n) \tag{2}$$

where a_1 and a_n are the first and the *n*-th value of absorbance (a). After, autoscaled absorbance (b) for each value of a was determined by

$$b = \frac{a - a_{min}}{a_{max} - a_{min}} \tag{3}$$

In order to avoid the calculation mistakes resulting to scaling from 0 to 1, the zero absorbance was approximated by the calculation of average value between two adjacent values of absorbance satisfying that $b_{j-1} < b_j < b_{j+1}$ where $b_j = 0$.
Since derivative spectrum is strongly sensitive to noise in the original signal, the smoothing of noise was decreased by the use of average-based spectral filter (ABSF) (Palencia, 2018). ABSF is given by

$$ABSF(b_i; N = 20) = \frac{1}{3}\sum_{j}^{j+2}(b_j)\Bigg|_{N=1}^{N=20} \tag{4}$$

ABSF is the moving average with a data window of 3 and 20 cycles (N = 20). As function is modified by the use of Equation 3, the same transformation of data is performed on function domain in order to correct small displacements respect to original spectrum.

In order to apply the FEDS transform, Function P must be calculated for each data of autoscaled spectrum. Details of Function P and FEDS have been previously published (Palencia and Martínez, 2017; Palencia, 2018). Function P is defined to be

$$FEDS = \frac{(1 + a_N)}{\sqrt{|p|}} \quad with\ p = \frac{1}{df_a} \tag{5}$$

where $(1 + a_N)$ is only an amplification factor for the assignation of a weight congruent with absorbance intensity and df_a is the derivate of f_a which is defined to be the spectrum line function. Note that, strictly speaking, equations have not limitations related with the technique, in consequence, equations should be useful for the analysis of any spectra or spectrum-like function. In order to analyze the repeatability of spectra, four replicates were performed for two samples. Later, by Pearson correlation coefficient (R^2) was evaluated the spectral similarity for pairs of spectra. Thus, test of similarity based on Pearson coefficient was based on the use of correlation matrix. Thus, for four replicates a matrix 4x4 is obtained. From the analysis of correlation matrix can be concluded which replicates can be averaged and therefore selected for analysis. After to determine what replicates, in a same set of data for a single sample, a comparative analysis between samples of different placed of fish body was performed. For that, two correlation matrixes were performed: 2 x 2 (from the average of samples with 4 replicates) and 4 x 4 (from the average of samples with 4 replicates and two samples with only one analysis).

3. Result and conclusions

3.1. Repeatability and reproducibility of analysis

In the Figure 1A and 1B are shown the spectra of replicates of two samples 1 and 2 which were analyzed 4 times. It can be seen that the repeatability is not complete because for the first sample one atypical spectrum can be identified whereas, for sample 2, two spectra are seen to be atypical. The above demonstrate that samples should be analyzed at least in quadruplicate in order to depurate and avoid mistakes. Therefore, for the analysis of biological tissue, in particular, fish scales, some strategy for spectrum discrimination and/or selection should be applied. Here we proposed the use of correlation matrix based on Pearson correlation coefficient (R^2). Thus, for correlation coefficients lower than 0.8 the spectra should be carefully revised. In the Table 1, correlation matrixes for sample 1 and sample 2 are shown, where it is evidenced that repeatability of analysis can be low, and with high risk to negatively affect the result when the analysis.

When correlation matrix is used, note that, for n = 4 spectra, being n the number of spectra, which can be denoted to be 1, 2, 3 and 4, different correlation pairs could be defined and, in consequence, a matrix 4x4 is easily made. The total number of R^2 (N_{R2}) that should be analyzed are:

$$N_{R2} = \frac{1}{2}(n^2 - n) = \frac{n(n-1)}{2} \tag{6}$$

The above equation can be used in order to performed an experimental design and algorithms of calculation. Illustration of correlation analysis for samples is shown in the Figure 2.

The differences in the spectra could be a result of analytical mistakes related with different factors,

Table 1. Correlation matrixes for sample 1 and 2.

	Sample 1				Sample 2			
	1	2	3	4	1	2	3	4
1	1.0000	0.9976	0.9969	0.7415	1.0000	0.4724	0.6041	0.4644
2		1.0000	0.9986	0.7611		1.0000	0.9505	0.5478
3			1.0000	0.7677			1.0000	0.6985
4				1.0000				1.0000

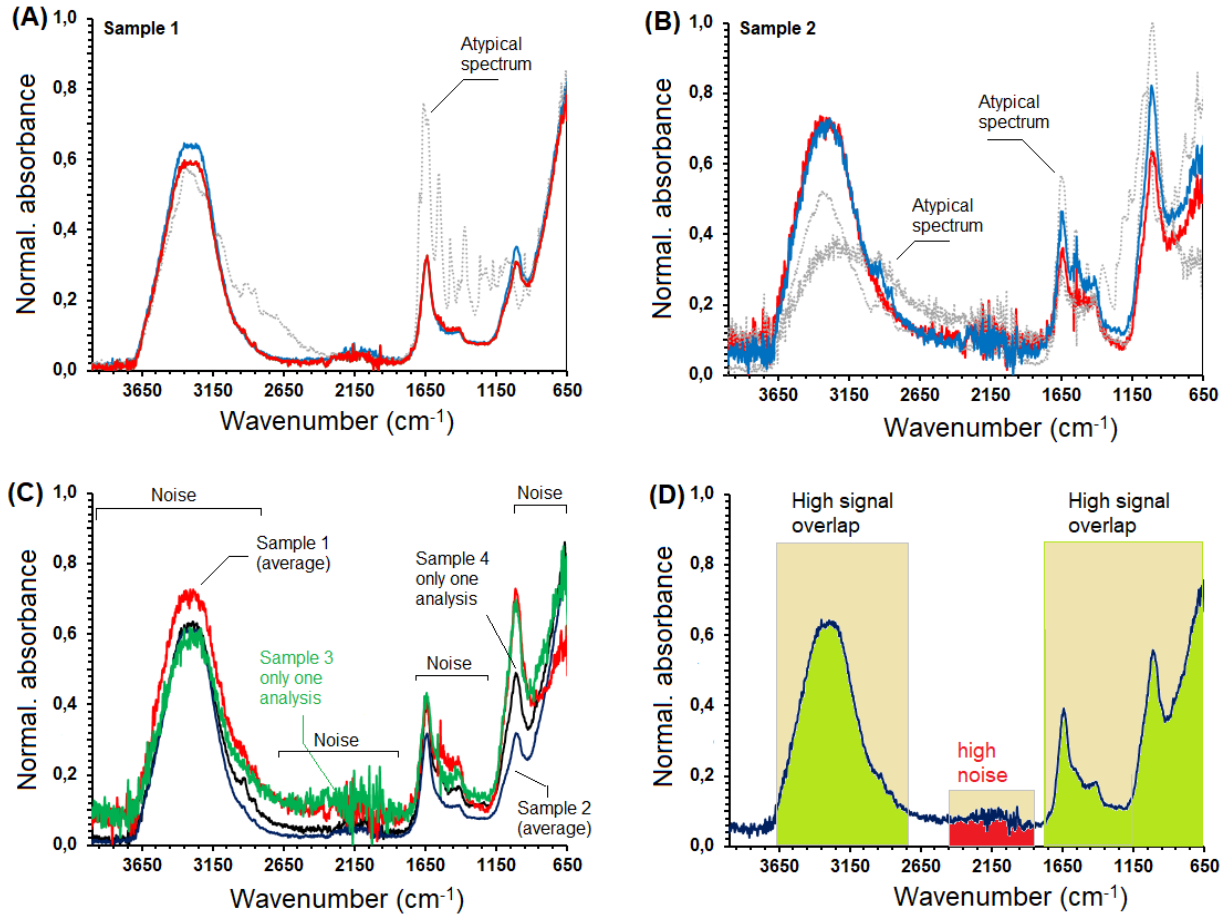

Figure 1. Spectra of FTIR of fish scale: (A) sample 1, (B) sample 2, (C) average samples 1 (red) and 2 (dark blue), and sample with only one analysis: samples 3 (green) and 4 (black), and (D) identification of high overlap into average spectrum.

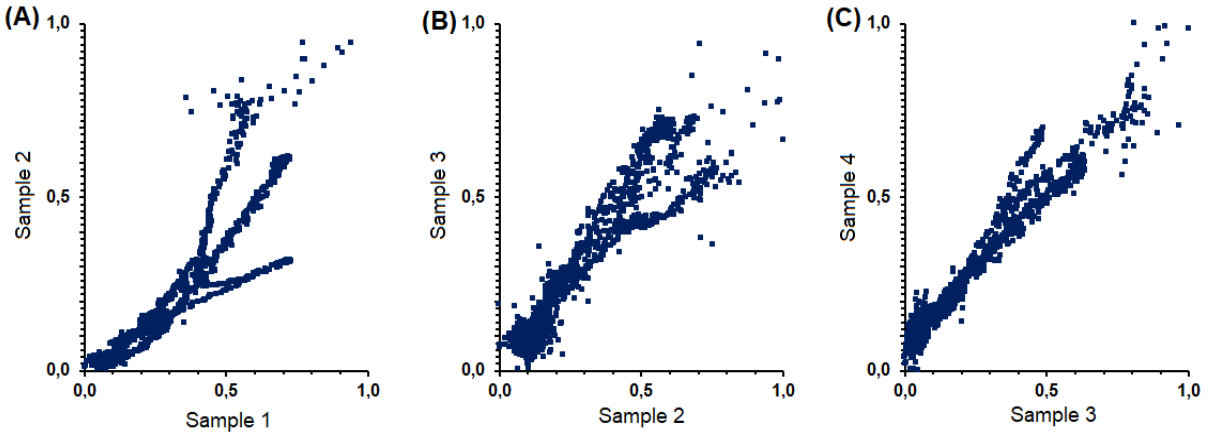

Figure 2. Correlation analysis for several samples of fish scale: sample 1 and 2 correspond to average spectra while sample 3 corresponds to one spectrum obtained from one single analysis.

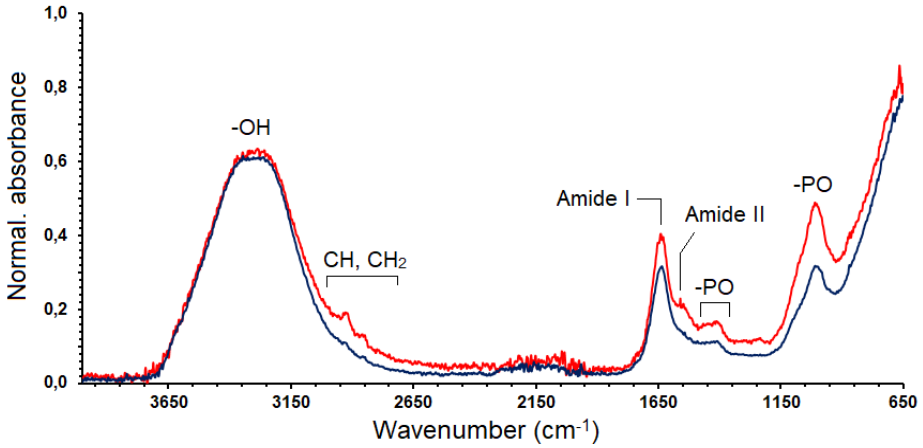

Figure 3. FTIR spectra with low end-spectrum noise.

e.g., poor contact between the sample and the ZnSe crystal, electrical current fluctuations, etc. In the Figure 1B and 1C can be observed great amount of spectral noise. In order to achieve a correct contact, it is suggested the pretreatment the samples by: (i) obtaining the dots, (ii) obtaining of sheet-type samples. Finally, in the Figure 1D, it can be seen that a high overlap is obtained during analysis meaning that obtaining of information is restricted when the analysis is based only in the FTIR spectrum.

3.2. Infrared characterization of fish scale of *Prochilodus reticulatus*

FTIR spectra with lower noise are shown in the Figure 3. It can be seen that typical signals can be identified: at 3300 cm^{-1} is observed vibrations of -OH of water, which clearly it is overlapped with signals associated with C-N of amide, however, this is not identified. Between 2750 and 2900 cm^{-1} are expected vibrations of CH and CH_2. Two signals can be associated with amide I and amide II of collagen molecule and vibrations of -PO associated with hydroxyapatite. However, by FTIR only two signals are identified to be important: Amide I (~1640 cm^{-1}) and -PO (1000 cm^{-1}).

3.3. FTIR+FEDS analysis of spectrum of fish scale of *Prochilodus reticulatus*

FEDS analysis of FTIR spectrum of fish scale of 'bocachico' or *Prochilodus magdalenae* is shown in the Figure 4. In this figure, the spectrum analysis is shown from 1900 to 4000 cm^{-1} whereas analysis from 600 to 1900 cm^{-1} is shown in the Figure 5. In the Figure 4A can be seen the correlation of main signals of FTIR spectrum versus FEDS spectrum. However, it is evidenced that new signals appear as result of FEDS transform. Between 3000 and 3600 cm^{-1} can be identified signals associated with asymmetric and symmetric stretching of water molecules. It is important to indicate that FEDS intensity is not directly related with absorbance or concentration of groups on the material, in consequence, the small size of asymmetric stretch doesn't mean that the population of groups with asymmetric vibrational mode is larger than groups with symmetric vibrational modes. Signal at 3350 cm^{-1} is associated with -NH vibrations into structure of amino acids as proline and hydroxyproline (see Figure 4D). But also, signals between 3260 and 3280 cm^{-1} are associated with -NH into amino acids, mainly glycine and alanine (see Figure 4D). Amino acid composition of fish collagens has been described to contain high percentages of glycine, alanine, proline, hydroxyproline, glutamic acid and aspartic acid (Piez and Gross, 1960). However, assignation of proline should carefully revised since according Thuy et al., (2019) main amino acids extracted from fish scale (carp) are threonine (39.8 %), proline (11.4 %), glutamic acid (12.8 %), arginine (9.9 %), serine (8.3 %), alanine (5.1 %), glycine (3.3 %), aspartic acid (2.3 %), cysteine (1.4 %), histidine (1.1 %) and lysine (1.0 %) with percentages lower than 1.0 % for other amino acids (phenylalanine, valine, isoleucine, methionine, hydroxyproline, among other) (Thuy et al., 2019).

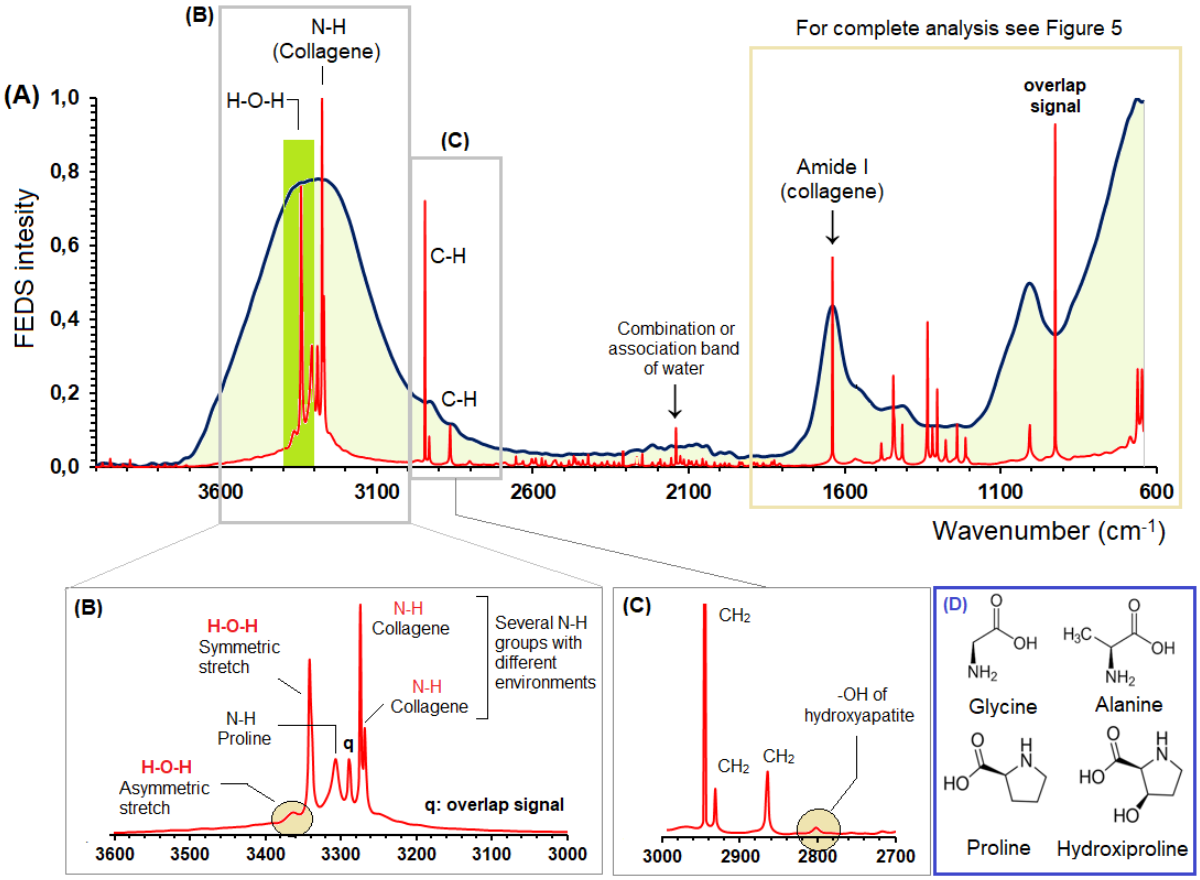

Figure 4. FEDS analysis of FTIR spectrum of fish scale: (A) comparison of FEDS spectrum versus FTIR spectrum, (B) analysis from 3000 to 3600 cm^{-1}, (C) analysis from 2700 to 3000 cm^{-1}, and (D) structure of important amino acids existing in the collagen.

Signal denotes as 'q' corresponds to overlap signal and is resulting of adjacent signals overlapped. In this analysis window the applicability of FEDS is demonstrated because coherent signals were obtained from deconvolution of wide band initially associated only with -OH. But also, two important components of fish scale are identified by these characteristic vibrations: water and collagen (Ikoma et al., 2003; Gil-Duran et al., 2016). In the first case, in addition to signals associated with -OH, there is a small but significant combination band (IR and Raman) of the bending and oscillation or vibration modes at ≈ 2125 cm^{-1}. This band, also known as the 'association band', may be due to the third overtone of the oscillation band, with the second overtone introducing asymmetry into the bend vibration. Other vibrations can contribute, such as a combination of bending and oscillation, and intermolecular interactions (Millo et al, 2005).

Between 2700 and 3000 cm^{-1} are observed characteristic vibrations of CH_2 and CH links which are associated with organic phase of fish scales, exactly, collagen (see Figure 4C). But also, a small signal at 2800 cm^{-1} is associated with -OH signals of hydroxyapatite (Gheisari et al., 2015). This signals also is coherent with the composition of fish scales (Ikoma et al., 2003; Gil-Duran et al., 2016).

Analysis by FEDS of fish scale spectrum from 600 to 2000 cm^{-1} is shown in the Figure 5. By review of publications can be identified signals associated with hydroxyapatite: PO_4^{3-} (at 1000 cm^{-1} and 960 cm^{-1} being intense and weak signals, respectively), HPO_4^{2-} (~870 cm^{-1} which is associated with hydroxyapatite deficient of calcium and non-stoichiometric structure), -OH (at 685 cm^{-1}) and PO_4 (at ~650 cm^{-1}) (Gheisari et al., 2015; Berniza-Cimdina and Borodajenko, 2012). Note that overlap signals at 920 cm^{-1} permits to conclude that two signals exist around this signal, one below and one above in wavelengths.

On the other hand, it is clear that other signals should be associated with collagen. In particular,

since collagen is a polypeptide is important identify the signals associated with the peptide nature, thus: Amide I (C=O + C-N) at 1640 cm^{-1}, amide II (C-N + N-H bending) at 1550 cm^{-1} and amide III (N-H band + C-C stretch + C=O bend) at 1480 cm^{-1}, but also, C-N stretching at 1210 cm^{-1} can be identified, which have been previously described for surface of polyamides (Zarshenas et al., 2015). An illustration of segments of collagen is shown in the Figure 5C evidencing its molecular complexity.

Signals associated with proline have been described at 1242 cm^{-1} (stretching of C-O; 1240 cm^{-1} in this study), 1292 cm^{-1} (in-plane bending of CH; 1280 cm^{-1} in this study), 1320 cm^{-1} (torsional mode for CH_2; the same value in this study), 1375 cm^{-1} (in-plane bending of OH; it is not identified, but it suggested that signal is displaced at 1420 cm^{-1} because oxygen can strongly interact by hydrogen links) and 1446 cm^{-1} (in-plane bending of CH_2; the same value in this study) (Sheena et al., 2009). Finally, signal named as 'b' corresponds to overlap signal.

In addition, it is suggested that small vibrations between 690 and 810 cm^{-1} are associated with characteristic vibrations of collagens, e.g., backbone of biopolymer, or out-of-plane C-H bend of polyamides have been described to appear at ~800 cm^{-1} (Gabelich et al., 2005).

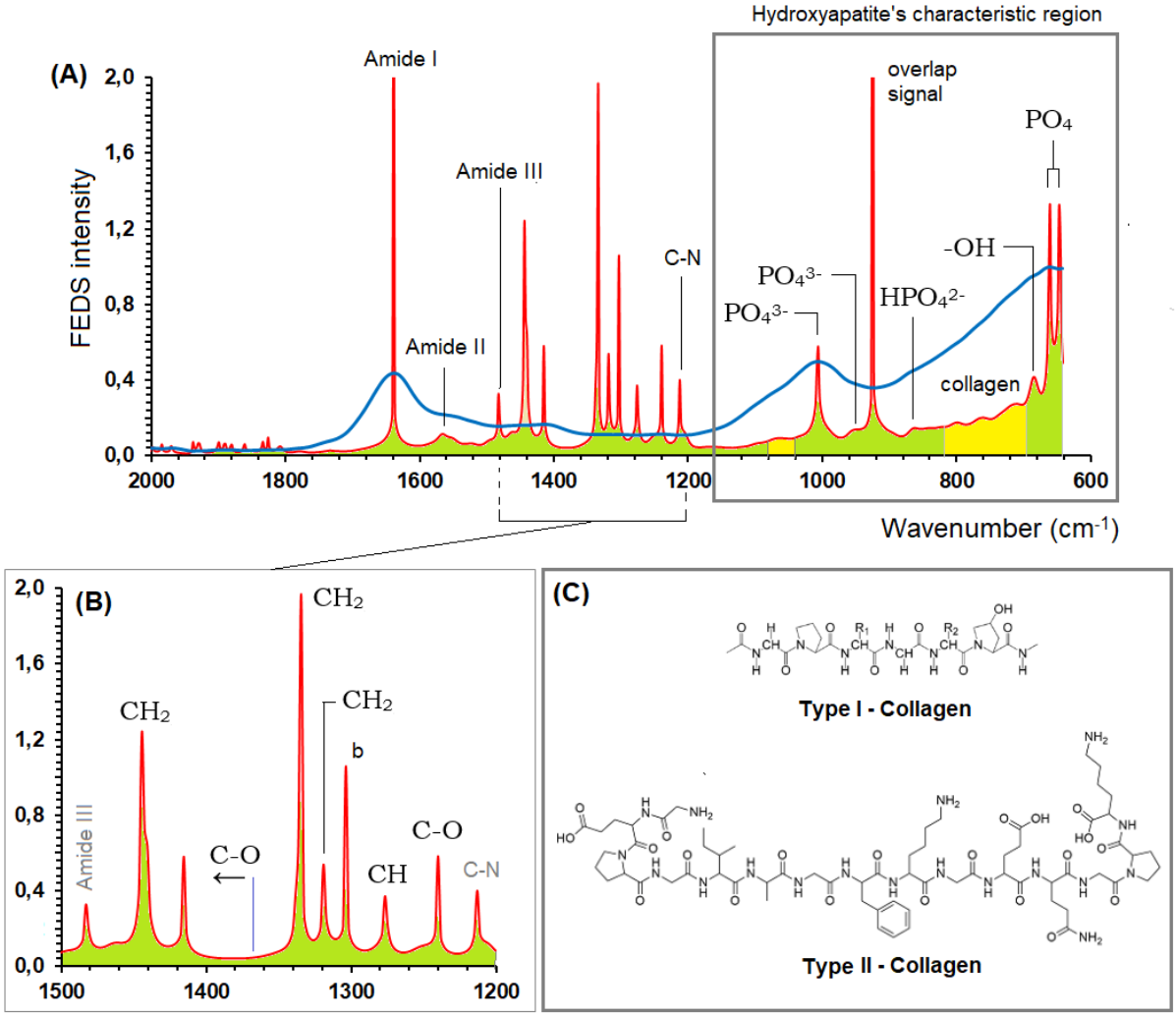

Figure 5. (A) FEDS analysis between 600 and 2000 cm^{-1}, (B) FEDS analysis between 1200 and 1500 cm^{-1}, and (C) illustration of collagen structure.

3.4. Remarks about analytical protocol for FTIR+FEDS analysis of fish scale samples

For the performing of FTIR+FEDS analysis of fish scale samples it is suggested the performing of following protocols:

• The performing of correct sampling at level of population (when the research is directed to analysis of populations or environmental aspects).

• The performing of correct sampling at level of individuals. Fish scale should be selected on both sides of the body of the fish, trying to cover both the middle areas and the ends of the body, avoiding edges too close to the fins. Although a specific effect has not been studied, the results of the repeatability and reproducibility analyzes in this study show that the technique can be very sensitive, and therefore, greater uniformity in the scales is desired. The minimum number of suggested samples is four.

• Depending of objective, the pre-treatment of samples is performed: (i) in order to analyze the presence of substance which can adsorbed on surface of scales, it is suggested the surface cleaning with water, dried at 45 °C and subsequently the analysis. (ii) In order to analyze the composition of fish scale a pre-treatment warranting the elimination of substance exogenous adsorbed should be performed.

• Analysis by ATR must be carried out increasing the contact of fish scale with surface of crystal. For that, samples can be reduced to dots when only it is desired explore the composition. However, lamellar-type samples can be useful in order to analysis surface differences. Note that, when scale is analyzed directly, putting the external surface in contact with ATR crystal, the noise is increased but also additional analysis can be performed, e.g., penetration deep of IR ray can be used to study the molecular density of fish scales.

• For the selecting of spectrum obtained from one same sample, it is suggested the use of correlation matrix and characterization of end-spectrum noise. The same is suggested for the selecting of sample groups.

• Smoothing of spectrum should be performed after to correlation analysis. In general, 20 cycles of smoothing using a window of 3 consecutive data are suggested, the above is based in previous works.

• In order to achieve an objective assignation of signals, samples of compounds are required to be used as reference, or data base of FEDS signals. Assignation based only in data base of infrared spectra is very difficult and inexact. However, studies of vibrational properties of components could be very useful.

4. Conclusions

It is concluded that FTIR spectroscopy in conjunction with FEDS transform is a promissory technique for the analysis of biological surfaces of interest for aquiculture science and technology. In particular, for fish scale this technique can be used to study of surface composition by analysis of functional group vibrational modes using ATR technique. Thus, it is concluded that for a correct analysis and warrant an adequate reproducibility of recorded information, samples should be analyzed at least by quadruplicate, spectra should be selecting by correlation analysis method or another methodology and finally averaged. In addition, the end-spectrum noise should be characterized in order to be used as selecting criteria and smoothing technique can be used for minimization of residual noise. On the other hand, it was demonstrated that FEDS transform can be used to analyses the line function of spectrum and perform the deconvolution of signals in order to achieve a significant improving into the analysis.

Interest conflict

The authors declare no conflict of interest.

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