Identification of composition and structure of functional groups of ferment lysates based on IR spectroscopy

O P Dvoryaninova¹, A V Sokolov¹, O V Peregonchaya², E A Solovyeva³ and D A Syanov³

¹ Voronezh State University of Engineering Technologies, 19, Revoljucii Ave., Voronezh, 394036, Russian Federation
² Voronezh State Agrarian University named after Emperor Peter the Great, 1, Michurina str., Voronezh, 394087, Russian Federation
³ Moscow State University of Technologies and Management named after K.G. Razumovsky, 73, Zemlyanoy Val, Moscow, 109004, Russian Federation

E-mail: olga-dvor@yandex.ru

Abstract. The paper provides the analysis of IR spectra of ferment lysates, which showed that there are a number of weak bands 1050-1150 cm⁻¹ in spectra typical for vibrations of ester bonds and frequency 1150-1300 cm⁻¹ corresponding vibrations of CH₂ groups in fats. Given the high intensity of the peaks at 2800-3000 cm⁻¹, the high intensity of the peaks at 1600-1700 cm⁻¹ and 1500-1550 cm⁻¹ (typical for amino acids) can be assumed to be high in amino acids containing NH₃, СН₂ and CH₃ groups. The study showed that hydrolysis products (ferment lysates) derived from secondary fish processing products have similar protein structure and identical peptide fragments.

1. Introduction
The structural-functional state of proteins at phase boundaries largely determines the course of many vital processes in cells. It is at the phase boundaries that many biomolecules responsible for transport, metabolism and signal transduction processes are localized and functioning. Thus, the development of new approaches to the study of structural-functional features of proteins and enzymes in model colloidal systems presents particular interest from a fundamental point of view. Besides, molecular study of mechanisms of formation and stabilization of artificial colloidal systems is also important for the development of new technologies for food and feed industry [1, 2, 9, 10].

At present, there are practically no direct methods for studying the structural properties of proteins adsorbed on water-air and water-oil interfaces. The information on the structural properties of proteins is extremely rare in the literature and is rather qualitative. There are almost no approaches to monitor changes in the structure and aggregation state of proteins during real-time adsorption [3, 4].

One of the available and relatively simple from the methodical perspective, and at the same time extremely informative methods for analyzing the structure of biomolecules, including in nanobiosystems, is the Fourier-transform infrared spectroscopy. The method is based on the registration of vibrational spectra of molecules, which gives detailed information on the state of molecules or functional groups in the sample under study. IR spectroscopy is a highly sensitive physical method that allows rapid and accurate analysis of the structure of biomolecules (proteins, lipids, polysaccharides and...
other biopolymer molecules) when studying several micrograms of the substance. IR spectroscopy is now a common method of studying the secondary structure of proteins at the quantitative level. IR spectroscopy is widely and successfully used to study the structure of proteins included in both homogeneous and various heterogeneous and microheterogenous systems. The feature of IR spectroscopy is that it does not require optical transparency of the solution, which makes it possible to measure protein spectra in large membrane fragments, in suspensions, in immobilized aggregated state, in inclusion bodies, etc. A unique advantage of IR spectroscopy is the ability to study the structure and spatial orientation of biomolecules in intact biological membranes or in artificial lipoprotein complexes – protein-containing liposomes. This makes it possible to analyze the structure of many membrane proteins that cannot be studied by X-ray analysis. This method is promising for investigating the structure of proteins that constitute different nanobiosystems. Thus, IR spectroscopy was successfully used to study the structure of supramolecular protein complexes in the formation of peptide nanotubes; as well as the structures of proteins incorporated into the liposomes and reversed surfactant micelles; to analyze the enzyme structure in the composition of enzyme-polyelectrolyte complexes, both in soluble and gel state. An important advantage of IR spectroscopy is the ability to monitor intermolecular interactions in proteins or aggregation. Aggregation is one of the main mechanisms of enzyme inactivation and plays a key role in gelation, as well as in the processes of self-assembly of proteins into supramolecular complexes (including the formation of nanostructures, nanospheres and nanotubes). Therefore, the ability to directly monitor protein aggregation is essential to study the properties of enzymes and protein-containing systems [5, 6].

2. Materials and methods

The samples of ferment lysates obtained from the secondary processing products (SPP) of pink salmon and silver carp were placed in a multiple disturbed attenuated total reflection console by Pike Technologies™. IR spectrum was taken using Fourier Transform IR spectrometer InfraLum FT-08 in the area of 600 – 4000 cm⁻¹. Measurement conditions: number of scans – 93 per minute, spectrum resolution – 1 cm⁻¹.

3. Results and discussion

The IR spectra of ferment lysates of pink salmon and silver carp obtained from fins, skins and scales revealed that there were a number of weak bands of 1098, 1120 cm⁻¹ typical for ester bond vibrations in the spectra (Figures 1-3). The frequencies 1180, 1261 cm⁻¹ correspond to vibrations of CH₂ groups in fats. Thus, it can be assumed that there is a small amount of fat in the sample. The areas of 1300-1339 cm⁻¹ and 1560-1600 cm⁻¹ demonstrate the stretching and bending vibrations of NH₃ groups in amino acids or amines included in proteins. The frequencies of 1695-1730 cm⁻¹ are set for carboxyl groups in amino acids. The areas of 1300-1339 cm⁻¹ and 1560-1600 cm⁻¹ are typical for vibrations of a COO group fragment in a carboxyl group. The peaks of 1397 and 1430 cm⁻¹ correspond to vibrations of NH₂ group in amines in ionized state. The 1458 cm⁻¹ band corresponds to vibrations of OH groups in amino acids. The areas of 1485-1550 cm⁻¹ and 1610-1670 cm⁻¹ demonstrate the stretching and bending vibrations of NH₃ groups in amino acids or amines included in proteins. The frequencies of 1695-1730 cm⁻¹ are set for carboxyl groups in amino acids. The peaks of 2849 and 2917 cm⁻¹ are typical for CH₂ and CH₃ groups in fats, amines and certain amino acids (alanine, valine, leucine, isoleucine, threonine). Given the high intensity of peaks 2849 and 2917 cm⁻¹, 1538 cm⁻¹ (typical for amino acids) and the low intensity of peaks 1098 and 1120 cm⁻¹ (typical for fats), the high content of amino acids containing CH₂ and CH₃ groups can be assumed.
Figure 1. IR spectra of ferment lysates from fins: a – pink salmon; b – silver carp

Figure 2. IR spectra of ferment lysates from scales: a – pink salmon; b – silver carp
The areas of 3030-3500 cm\(^{-1}\) contain many vibrations typical for NH\(_2\) and OH groups in amines and amino acids.

The area of more than 3500 cm\(^{-1}\) contains combined frequencies and is low-informative for analysis.

Figure 3. IR spectra of ferment lysates from skin: a – pink salmon; b – silver carp

Spectrograms of samples taken in the range of wave numbers from 4000 to 800 cm\(^{-1}\) include two characteristic areas. Short-wave band (4000-2800 cm\(^{-1}\)) contains the bands of stretching vibrations of C-H, N-H and O-H bonds, position and intensity of which for all samples almost coincide. Absorption in the long-wave band of the electromagnetic spectrum (1800-800 cm\(^{-1}\)) characterizes the vibrations of bonds of atoms included in the functional groups of protein molecules, peptides, as well as low molecular weight products of fermentolysis.

Figure 4 shows IR spectra of pink salmon ferment lysates (Figure 4a) and silver carp (Figure 4b) obtained from fins (curves 1), scales (curves 2) and skin (curves 3). All studied samples demonstrate the presence of absorption bands typical for stretching and bending vibrations of atoms included in peptide groups: amide I (1650-1645 cm\(^{-1}\)), amide II (1545-1540 cm\(^{-1}\)), amide III (1275-1236 cm\(^{-1}\)).
Figure 4. IR spectra of ferment lysates: a – pink salmon, b – silver carp, obtained from: 1 – fins, 2 – scales, 3 – skin. Wave number range $\nu$ from 1800 to 800 cm\(^{-1}\).

The study proved that enzyme-catalyzed proteolytic hydrolysis of peptide bonds and formation of carboxyl and amino groups, as well as their salt forms at the point of rupture occurs during fermentolysis of protein molecules. The works [5, 6, 7, 8] show the correlation of spectral behavior of hydrolysate samples and the degree of their hydrolysis. The most sensitive to such transformations are the following: symmetric and asymmetric stretching vibrations of carboxylate ions exhibited on the spectrograms of the samples at 1440-1400 cm\(^{-1}\), 1580-1550 cm\(^{-1}\), respectively [4, 6]; bending vibrations of N-H bond in the composition of primary (1650-1580 cm\(^{-1}\)) and secondary (1600-1500 cm\(^{-1}\)) amino groups. The comparison of the intensity of absorption bands of terminal groups of peptide amino acid fragments with the intensity of the maximum amide I can be used to characterize the process depth and fermentolytic decay products [5, 6, 7].

The IR spectra of ferment lysates of pink salmon and silver carp (Figure 4 a, b) received from fins (curves 1) and skin (curves 3) show the band of 1432-1428 cm\(^{-1}\) corresponding to stretching vibrations of COO$^{-}$ carboxylate ions and methyl or methylene groups overlapped with the absorption maximum at the nitrogen atom participating in the formation of salts as part of$-\text{CH}_2-\text{NH}_3^+$ fragment [3]. The presence of this band is justified by the significant content of glycine, arginine and lysine in the amino acid composition of ferment lysates from fins and skin of both fish species. A distinctive feature of spectrograms of protein hydrolysates of pink salmon and silver carp fins (Figures 4 a and b, curves 1) is the presence of intense absorption bands in the range of 1576-1563 cm\(^{-1}\) corresponding to the vibrations of carboxylate ions and primary amino groups and caused by the presence of glutamic, aspartic acids and their salts in ferment lysates.

Figure 5. Ratio of peak heights $h_x / h_{1650}$ of IR spectra of ferment lysates of pink salmon and silver carp obtained from skin, fins (x = 1430 cm\(^{-1}\)) and scales (x = 1400 cm\(^{-1}\)).
Spectral behavior of pink salmon and silver carp is characterized by the displacement of vibration band of carboxylate ions up to 1400 cm\(^{-1}\) and the maxima of absorption of bending vibrations N-H in the composition of primary (1460 cm\(^{-1}\) for pink salmon ferment lysates) and secondary (1500 cm\(^{-1}\) for silver carp) amino groups (Figure 4 a and b, curves 2). This fact can be explained by the decrease in the proportion of free amino groups involved in salt formation. The absorption band intensity of terminal carboxyl groups of amino acid peptide fragments for scale samples (1400 cm\(^{-1}\)), skin and fins (1430 cm\(^{-1}\)) was compared with the absorption band height of amide I (1650 cm\(^{-1}\)) using a baseline method.

Figure 5 shows a comparison of the peak height corresponding to the vibrations of carboxylate ions (hx) with the height of the amide I band (h1650) for different samples.

| Table 1. Correlation of bands in the spectrum of ferment lysates |
|-----------------|------------------|
| Frequency, cm\(^{-1}\) | Functional group                                      |
| 1098            | Ester groups in fats                                    |
| 1120            | CH\(_2\) groups in fats                                  |
| 1180            | COO group as part of COOH                               |
| 1261            | NH\(_4^+\) group in amines                              |
| 1316            | OH groups as part of COOH group                         |
| 1339            | Stretching and bending vibrations of NH\(_4^+\) group as part of amino acids |
| 1397            | COO group as part of COOH                               |
| 1430            | Stretching and bending vibrations of NH\(_4^+\) group as part of amino acids |
| 1458            | C=O groups as part of COOH                              |
| 1620            | Mainly CH\(_3\) and CH\(_2\) groups in some amino acids (alanine, valine, leucine, isoleucine, threonine) |
| 1636            | C=O groups as part of COOH                              |
| 1649            | NH\(_2\) and OH groups as part of amines and amino acids |
| 1670            | Combined frequency (overtone)                          |
| 1697            | Over 3500                                               |
| 1716            |                                                           |
| 1733            |                                                           |
| 2849            |                                                           |
| 2917            |                                                           |
| 3030 – 3500     |                                                           |
| 3500            |                                                           |

Thus, the hydrolysis of protein feedstock of pink salmon and silver carp scales results in relatively fewer terminal carboxyl groups than the number of amide bonds in peptide chains. In turn, fin and skin
ferment lysates are characterized by higher carboxylate ion content compared to peptide bonds. Besides, in case of pink salmon the hydrolysis takes place with the formation of more number of end fragments.

The correlation of bands in IR spectra of ferment lysates to functional groups are shown in Table 1.

4. Conclusion

The findings suggest deeper hydrolysis of protein chains for pink salmon fins and skins of both fish species than in the case of fermentolysis of their scales. However, the average molecular weights of peptides tested by ferment lysates cannot be estimated by simply comparing the intensity of the characteristic absorption bands.

The studies showed that hydrolysis products (ferment lysates) derived from the SPP of pink salmon and silver carp have similar protein structure composition and the presence of identical peptide fragments.

Thus, the identification of substances and materials is a complex analytical task, which requires not only studying the characteristics of objects obtained using a set of analytical methods, but also taking into account the peculiarities of the origin of the object of analysis, sample preparation and storage conditions.

References

[1] Krasnikova L V and Gunkova P I 2014 Microbiological safety of food raw materials and finished products Study guide (SPb.: ITMO; IRBT)

[2] Redkin N A 2019 IR-Fourier spectrometry and mass spectrometry in the identification of organic compounds: Study guide (Samara: Samara University publishing house)

[3] Kazitsyna L A and Kupletskaya N B 1979 Application of UV, IR, NMR and mass spectroscopy in organic chemistry (M.: Moscow State University publishing house)

[4] Glotova I A, Galochkina N A, Selemenev V F, Peregonchaya O V and Sokolova S A 2019 IR-spectroscopic study of immobilization of selenium compounds on biomodified collagen Periodico Tche Quimica 16 (33) 159-168

[5] Kristoffersen K A, Liland K H, Bocker U, Wubshet S G, Lindberg D, Horn S J and Afseth N K 2019 FTIR-based hierarchical modeling for prediction of average molecular. weights of protein hydrolysates Talanta 205 120084

[6] Poulsen N A, Eskildsen C E, Akkerman M, Johansen L B, Hansen M S, Hansen P W, Skov T and Larsen L B 2016 Predicting hydrolysis of whey protein by mid-infrared spectroscopy Int. Dairy J. 61 44-50

[7] Kudryashova E V and de Jongh H H J 2007 Modulation of the adsorption properties of the complex of egg white ovalbumin with pectin by the dielectric constant J. Coll. Int. Sci. 318 (2) 430-439

[8] Kudryashova E V, Visser A J W G, Hoek A and de Jongh H H J 2007 Molecular details of ovalbumin-pectin complexes at the air/water interface: a spectroscopic study Langmuir 23 (15) 7942-7950

[9] Dvoryaninova O P and Sokolov A V 2020 Productive feeding of rainbow trout: properties, effects on physiological state and interior indicators IOP Conference Series: Earth and Environmental Science (422) 012038

[10] Dvoryaninova O P and Sokolov A V 2018 Modeling of the drying process of secondary fish cutting products and description in the model of the main processes of heat and moisture transfer VSUET Bulletin 80 (2) 125-129