FT-IR AS A NEW TOOL FOR GUT ANALYSIS IN CULTURED FISH SPECIES.

Giorgia Gioacchini, Elisabetta Giorgini, Valentina Notarstefano, Gloriana Cardinaletti, Basilio Randazzo, Emilio Tibaldi, Ike Olivotto*

[1]Università Politecnica delle Marche, Dipartimento di Scienze della Vita e dell'Ambiente, Ancona, Italy. Email: i.olivotto@univpm.it

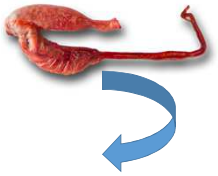
Introduction

It is known that the intestinal epithelium plays an important role in the absorption of nutrients osmotic balance, recycling of enzymes and macronutrients and its distal portion is the principal site for the endocytosis of proteins. Epithelial cells are also crucial mediators of mucosal innate and adaptive immunity; hence, a comprehensive understanding of the diet-gut interactions and immune-regulatory properties of intestinal epithelium could aid in the development of new strategies to prevent and treat infectious and inflammatory diseases in fish. Currently, there are many laboratory methods and analytical tools available for gut analysis, such as visual inspection and histology, biochemical, molecular and proteome analysis and, last but not least, microarray profiling and RNA sequencing (RNA-seq). However, these techniques and methods are normally expensive, time-consuming and laborious. Nowadays, there is a growing interest in exploiting the use of spectroscopic techniques for the study of biological samples, due to their high specificity, convenience, and quick response. Fourier Transform Infrared (FTIR) spectroscopy is a fast, label-free analytical technique, used for investigating functional groups, bonding types, and molecular conformations of the most relevant biological molecules. In order to develop knowledge on fish intestine and to complement morphological and molecular techniques currently applied in intestine quality evaluation, Focal Plane Array (FPA) FTIR imaging was applied to identify appropriate spectral biomarkers for seabream, seabass, and trout, three of the most important farmed fish species in the Mediterranean area.

Materials and Methods

Fish: reared at University of Udine aquaculture station.

Sampling



Histological analysis: intestine (foregut, pyloric caeca, midgut and hindgut intestine)

FTIR analysis: 10 µm thick sections of intestine samples (foregut, pyloric caeca, midgut and hindgut intestine), deposited, without any fixation process, onto CaF_2 optical windows.

Results

IR maps allowed to characterize for the first time the macromolecular composition of intestine sections of seabream, seabass and trout. A different macromolecular distribution in terms of proteins and lipids was found in all the analyzed sections. According to Seabream foregut, higher amounts of proteins were detected in muscle layer, while aspartate and glutamate were highlighted above all in the mucosa portion; *villi* resulted rich of lipids and triglycerides (Fig. 1). As concerns Seabass gut samples, a not homogeneous distribution of proteins and lipids was found in foregut, pyloric caeca and midgut portions (Fig. 2). Higher amounts of proteins were found in all gut portions of Seabass with respect to Seabream ones (data not shown).

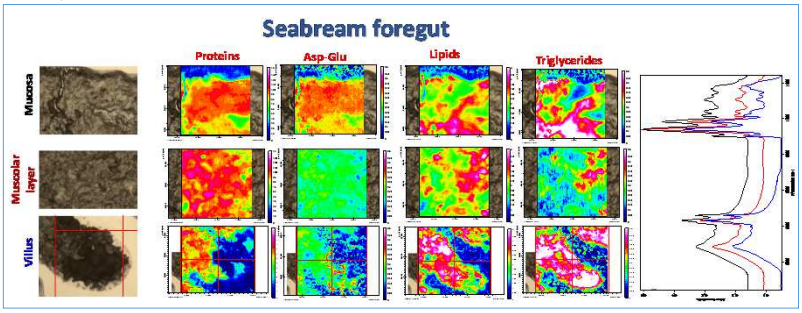


Figure 1

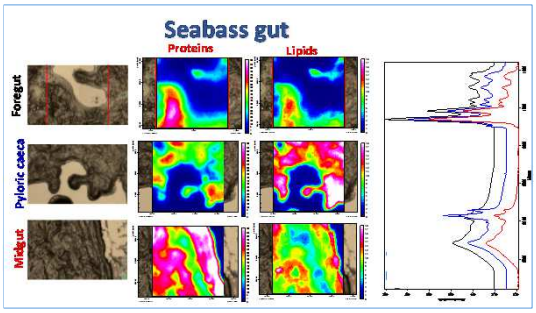


Figure 2

Discussion

By the preliminary but encouraging results already obtained in this study, the vibrational analysis of fish intestine appears a very promising approach in terms of quality evaluation. FTIR microspectroscopy can highlight subtle modifications in the spectral profiles of fish intestine, related to specific pathologies or to different diets. The huge number of spectral data contained in each IR map requires a further multivariate and statistical approach which is already in progress. In particular, a semi quantitative analysis is running to evaluate, in the intestine of various fish species, specific biological markers, related to lipid content and metabolism, proteins secondary structure, the occurrence of phosphorylative and oxidative processes and the degree of inflammation. All this information will let develop a new approach to better understand gut physiology in fish.

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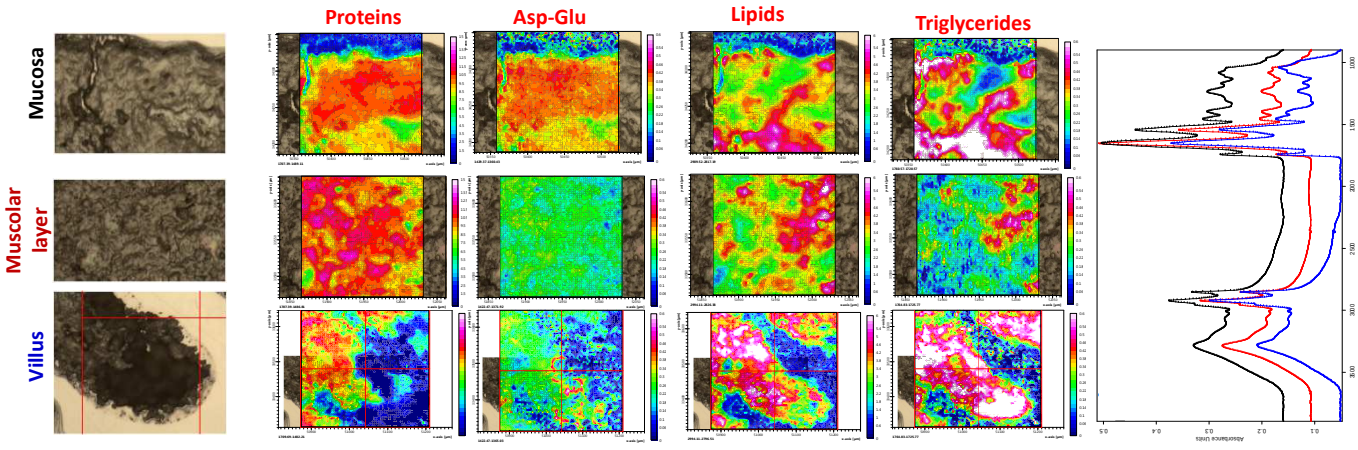
Seabream foregut



Seabass gut