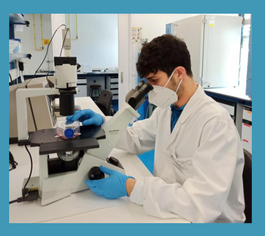
DETERMINATION OF THE SERUM PROTEINOGRAM PROFILE OF EUROPEAN SEABASS (*Dicentrarchus labrax*) FOR THE DIAGNOSIS OF FISH DISEASES



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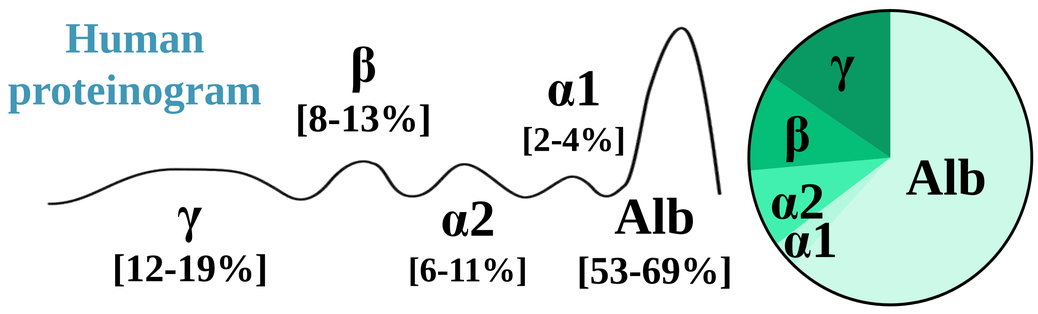
INTRODUCTION

A proteinogram is a semiquantitative analysis method that allows the separation of proteins by electrophoresis into different bands. This methodology has been widely used in clinical practice to detect the presence of abnormal or excessive proteins in serum, or other body fluids, which can be indicative of liver, inflammatory and immune disorders. Specifically, electropherograms have recently emerged as a specialization of protein electrophoresis in which the fluorescence intensities of proteins are measured as a function of their migration times. This method, widely used in humans, has not been studied in fish for diagnostic purposes.



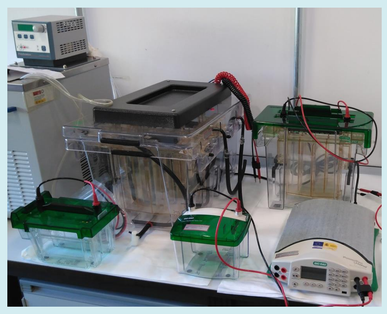
METHODOLOGY

European seabass (*Dicentrarchus labrax*)

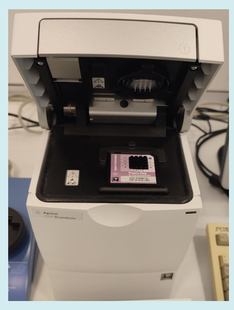
Serum

Serum protein quantification and adjustment

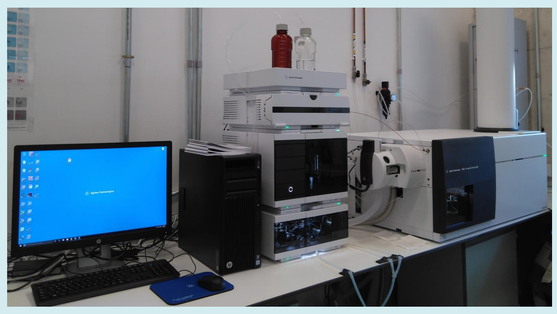
SDS-PAGE



Capillary electrophoretic Bioanalyzer



HPLC-Mass spectrometry



RESULTS AND DUSCUSSION

SDS-PAGE

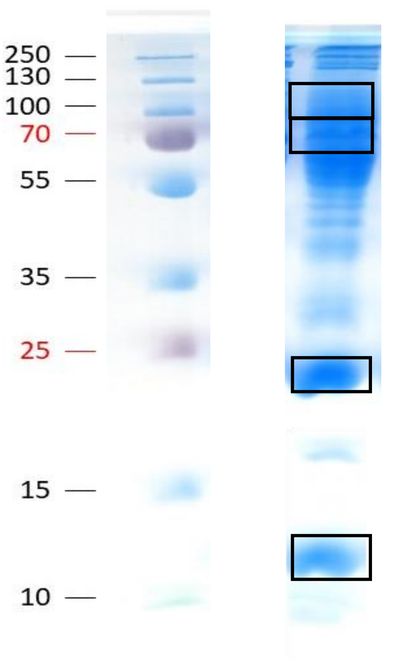
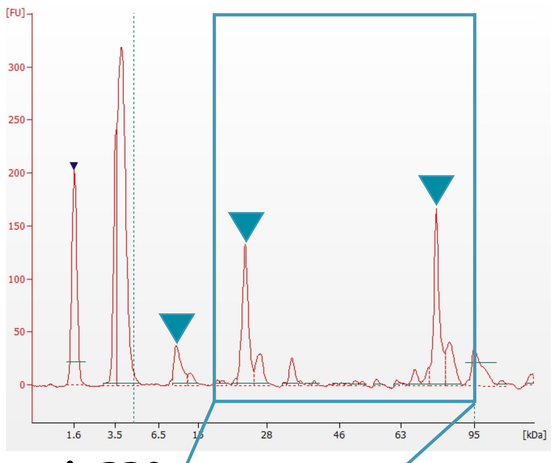


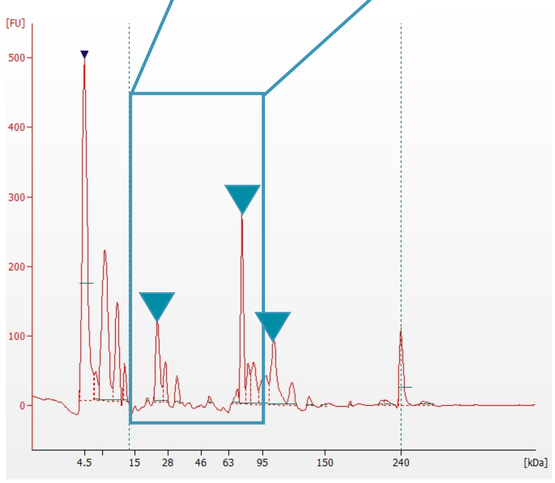
Figure 1. SDS-PAGE in sera of European seabass (*Dicentrarchus labrax*). The gels were stained with PageBlue Coomassie blue-based protein stain. Lines indicate where standards lie. The molecular weights (MW) are expressed in kilodaltons (kDa).

Electropherograms

A Protein80



B Protein230



Protein identification

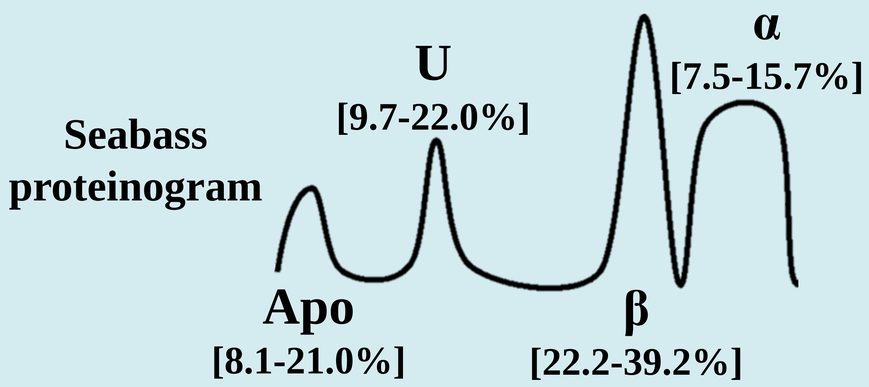
Electropherogram (Protein 80)			HPLC-Mass spectrometry	
Peak number	**Protein size [kDa]**	**Concentration (% of Total)**	**Protein size [kDa]**	**Protein**
1	**10.3**	**13.2**	**9.6**	**Apolipoprotein C-I**
2	12.3	4.7	12.4	Apolipoprotein C-II
3	18.6	0.8	19.2	C-type lectin domain-containing protein
4	19.6	0.8	19.7	C1q domain-containing protein
5	22.2	1.1	23.0	Apolipoprotein M
6	**23.9**	**27.0**	**23.9**	**Si:ch1073-126c3.2**
7	26.5	9.7	27.7	Complement C1q A chain
8	32.3	4.4	32.4	Apolipoprotein A-IV
9	36.3	0.5	36.5	Cystatin fetuin-A-type domain-containing protein
10	38.7	1.0	38.8	Cystatin fetuin-B-type domain-containing protein
11	46.7	0.7	47.4	Hemopexin
12	50.2	0.9	50.6	Vitronectin b
13	52.1	0.5	51.8	Angiotensinogen
14	55.8	1.1	55.1	Ig-like domain-containing protein
15	61.7	0.9	61.6	Complement component C8 beta chain
16	68.1	1.9	70.3	Prothrombin
17	**74.7**	**23.6**	**74.2**	**Serotransferrin**
18	83.7	7.2	83.4	Coagulation factor XIII. A1 polypeptide b

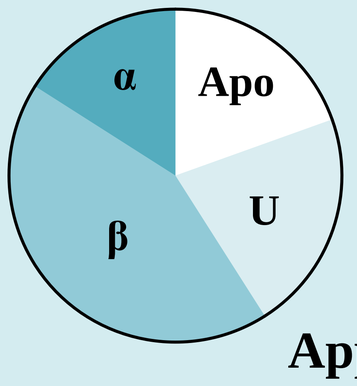
Electropherogram (Protein 230)			HPLC-Mass spectrometry	
Peak number	**Protein size [kDa]**	**Concentration (% of Total)**	**Protein size [kDa]**	**Protein**
1	20.8	1.7	20.8	Apolipoprotein M
2	**23.9**	**14.5**	**23.9**	**Si:ch1073-126c3.2**
3	27.2	5.8	27.7	Complement C1q A chain
4	33.1	5.0	32.4	Apolipoprotein A-IV
5	38.7	1.2	38.8	Cystatin fetuin-B-type domain-containing protein
6	51.7	1.3	51.8	Angiotensinogen
7	57.3	1.0	57.5	Serpin peptidase inhibitor. clade D member 1
8	71.1	3.6	70.3	Prothrombin
9	**75.5**	**29.1**	**74.2**	**Serotransferrin**
10	81.5	5.4	83.4	Coagulation factor XIII. A1 polypeptide b
11	87.6	7.7	87.4	Complement C2
12	93.7	4.2	93.1	Uncharacterized protein
13	97.9	3.6	99.1	Complement component 7a
14	**104.4**	**10.8**	**107.6**	**Inter-alpha-trypsin inhibitor heavy chain 2**
15	120.2	2.1	126.6	Ceruloplasmin
16	135.7	1.7	135.5	Immunoglobulin superfamily member 9B
17	148.5	0.5	147.6	Alpha-2-macroglobulin
18	219.5	0.8	212.4	Coagulation factor V

Figure 2. Electropherogram serum profile of European seabass (*Dicentrarchus labrax*) by Agilent Protein80 kit **(A)** and protein320 kit **(B)**. Peak migration times were compared to an external size standard (ladder; dark arrowhead) to determine size, while peak areas were compared to a lower and an upper marker to determine concentration. The accompanying tables provides an overview of each protein peak found by electropherogram with its size (kDa) and relative concentration (%) with respect to total protein. These protein peaks correspond to the size of a similar protein determined by high-performance liquid chromatography-mass spectrometry. The proteins with the highest concentrations are shown in bold type.

Conclusions

Reference values





Application in diagnosis of fish diseases

Acknowledgements:

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