Blood meal and blood products detection using Synchronous fluorescence spectroscopy

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Introduction

Context: The use of animal by-products (including processed animal proteins, **PAPs**) depends of their nature defined by the cell type and the species of origin. Currently, their detection is based on two methods: light microscopy and PCR. Complementary methods are needed to be developed in order to refine the by-products identification.

Objective: The aim of this work[1] was to develop a **fast and easy method** to detect blood meal and blood products. This study was based on the detection of hemoglobin in animal feed by **synchronous fluorescence spectroscopy (SFS).**

Materials & Method

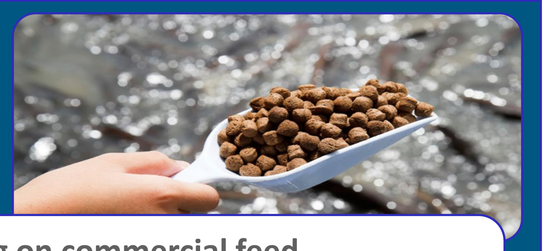
***Protein extraction by TCA/aceton precipitation following by resuspension in DLA[2]**

1. Optimization of the SFS conditions on reference materials:

- Hemoglobin powder
- Albumin powder

2. Validation on feed materials (protein extracts*):

- Hemoglobin powder (n = 8)
- Blood meal (n = 3)
- Plasma powder (n = 2)



3. Screening on commercial feed (protein extracts*):

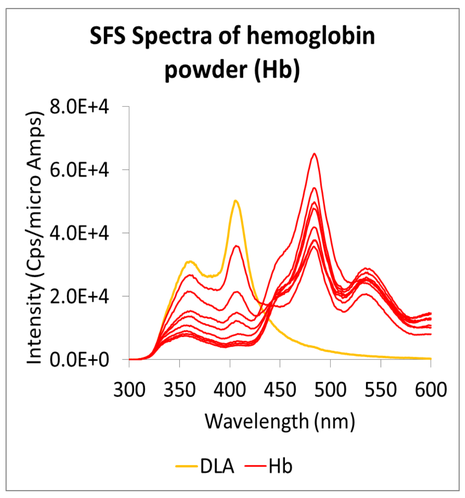
- Feed with plasma powder
- Feed with hemoglobin powder / blood meal
- Feed without any blood derived products

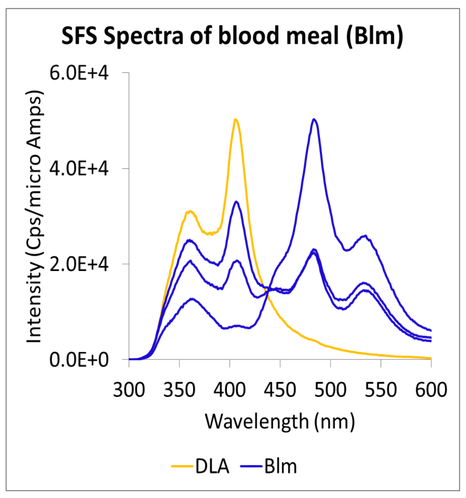
Results

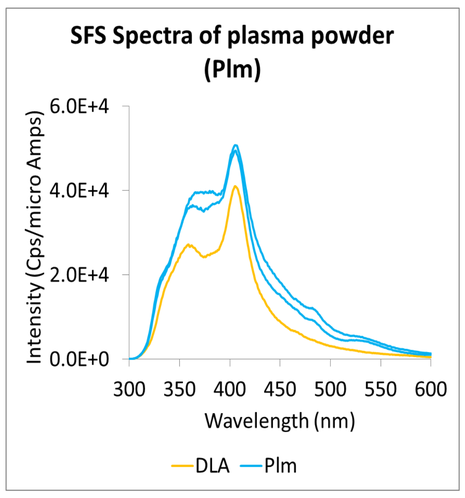
1. Optimization of the SFS conditions on reference materials:

Wavelength range	300 - 600 nm
Excitation/Emission slits	5.5 nm / 6 nm
Hemoglobin concentration	3 µM
Offset	17 nm
Solvent	DIGE Labelling Buffer (DLA)

2. Validation on feed materials (protein extracts*):

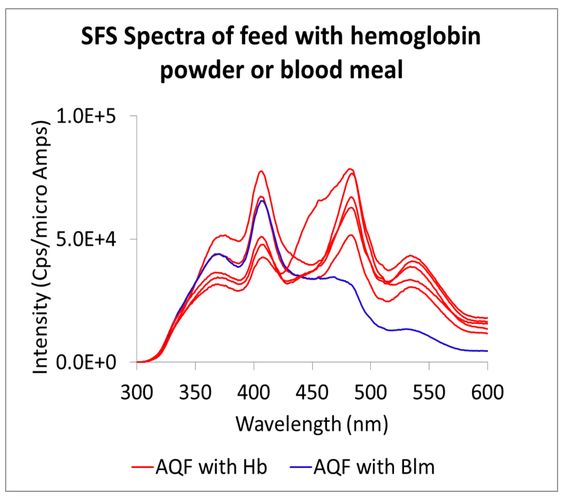


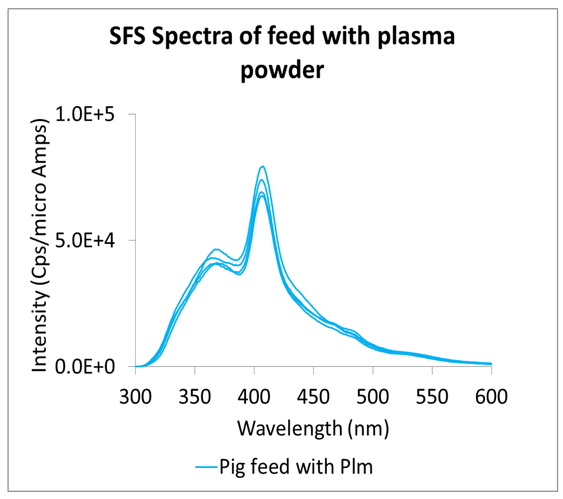


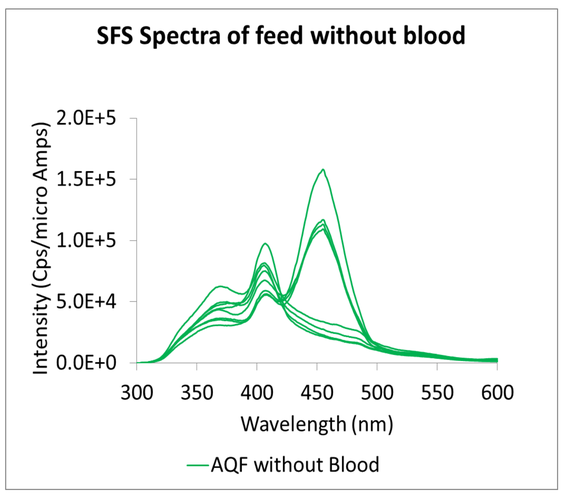


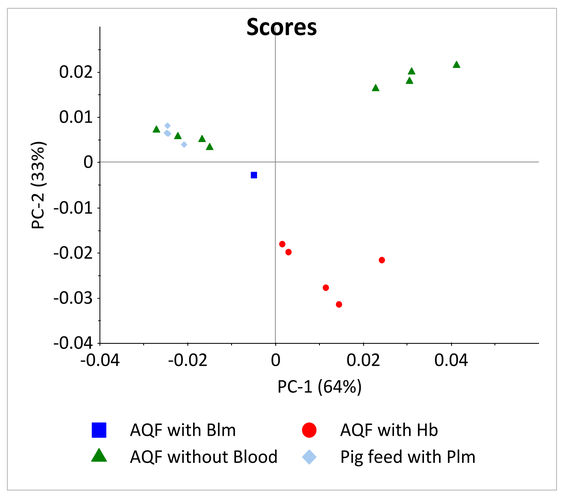
- DLA bands are present at 360 nm and 406 nm.
- Hemoglobin bands are present at 484 nm and 538 nm.
- Bands of hemoglobin are present in blood meal samples.
- Plasma powder doesn't contain hemoglobin. It has the same spectral profile as DLA.

3. Screening on commercial feed (protein extracts*):









- Hemoglobin spectral bands are present in feed known to contain hemoglobin powder or blood meal.
- Feed containing plasma powder don't show hemoglobin spectral bands.
- Feed without blood derived products don't present hemoglobin spectral bands; however four of them have an additional spectral band at 454 nm (unknown compound).

- Principal Component Analysis on normalized area SFS spectra allows the distribution of samples based on the presence of hemoglobin. Samples containing hemoglobin are located in the negative part of PC2 while the positive part of PC1 permits to distinguish two groups of feed without blood.

Conclusion et perspectives

Theses results confirmed that **SFS** is a promising **screening method** for the detection of hemoglobin in animal feed. Moreover, the method could also be used to evaluate hemoglobin extraction yield in support to other analytical methods.

References

[1] Taira, A. et *al.* (2016) Processed animal proteins detection using Fluorescence spectroscopy. Master Thesis .

[2] Lecrenier, M.C. et *al.* (2016) Identification of specific bovine blood biomarkers with a non-targeted approach using HPLC ESI tandem mass spectrometry. Food Chemistry, 213, 417-424.

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