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Differentiation of muscle fiber types by Raman spectroscopy (#615)

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Introduction

Raman spectroscopy has been shown to be a promising technology for the non-invasive determination of meat quality traits and for the characterization of muscle food. As the distribution of fiber types within the muscle is influencing the post-mortem characteristics of meat, it was interesting to assess whether Raman spectroscopy can be used for a rapid determination of muscle fiber types because conventional histology is time consuming due to the staining procedures. Muscle fibers are, in general, classified as slow twitch oxidative (STO, dark fibers), fast twitch oxidative (FTO, intermediate fibers) and fast twitch glycolytic (FTG, white fibers). It was hypothesized that the hem content of oxidative fibers can be used for the distinction from glycolytic fibers.

Methods

The *semimembranosus* muscle (SM) was chosen from five female pigs of German Landrace x Pietrain crossbreeds. The animals were commercially fed to slaughter weights of approx. 100 kg. Samples for staining and confocal Raman microscope scanning were taken at day 1 and 3 p.m. and they were snap frozen in isopentane cooled with liquid nitrogen. Serial cross sections of 12 µm thickness were cut with a cryotome. Some sections were stained for muscle fiber types using combined NADH-tetrazolium reductase/ATPase staining and adjacent non-stained cuts were used for Raman scanning. For each fiber type (STO, FTO and FTG) seven fibers were randomly selected and five Raman spectra were recorded at different positions with a confocal Raman microscope (LabRam HR, Horiba) with 532 nm excitation using resonance Raman scattering. In this way, 35 Raman spectra were obtained per fiber type and animal.

The potential for a differentiation by Raman spectra was further analyzed with principal components analysis (PCA) and partial least-squares discriminant analysis (PLS-DA) using MATLAB 7.9.0 R2009b software (The Mathworks Inc., Natick, MA, USA) and PLS toolbox 6.2 (Eigenvector Research Inc., Wenatchee, WA, USA).

Results

The results show differences in the Raman spectra between the three fiber types based on the intensities of characteristic hem signals (marked by arrows, Figure 1, averaged spectra per fiber type). As expected, the intensities of these signals were on average highest in the oxidative fibers (STO and FTO) and lowest in glycolytic fibers (FTG). However, PCA revealed that variance between animals had a large impact on the spectra. STO fibers could be discriminated to some extent from FTG fibers based on hem signals, but a clear discrimination of FTO fibers was not feasible using PLSDA.

Conclusion

The results confirm that resonance Raman spectra with excitation in the green spectral range can discriminate oxidative fibers from glycolytic fibers based on their hem signals. However the differences in the hem signal intensities (i.e. the hem content) between the three fiber types was not large enough or too variable for a clear discrimination of FTO fibers. Reasons are the bias by the animal and the limited number of spectra collected per fiber type. Further analysis of the spectra should focus on a larger number of fibers/positions so as to account for the variance in the muscle and also include other molecular constituents which could be useful for differentiation between the different muscle fiber types. As these Raman signals are to a large extent masked by hem signals in this experiment a non-resonant excitation wavelength could be useful to evaluate this

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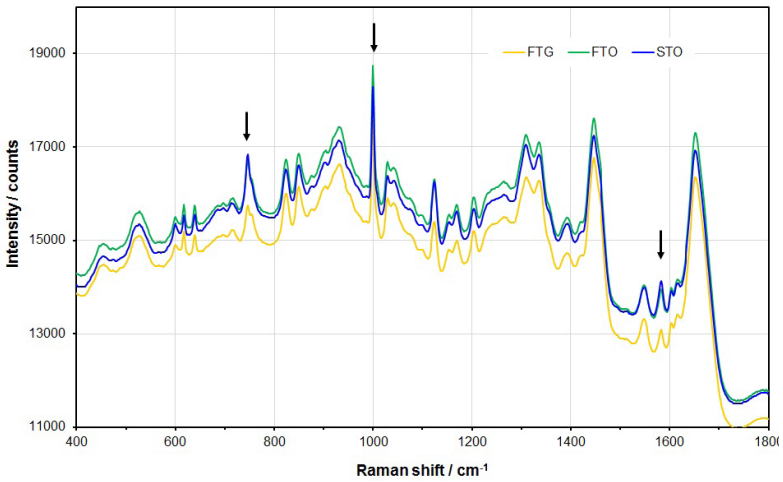

Figure 1 Averaged Raman spectra for fast twitch glycolytic (FTG), fast twitch oxidative (FTO) and slow twitch oxidative (STO) muscle fibers in *m. semimembranosus*; arrows indicate selected hem signals

Notes