PROTOCOLS EVALUATION FOR SERUM METABOLOMICS ANALYSIS IN BEEF CATTLE

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I. INTRODUCTION

Meat tenderness is traditional evaluated on meat after slaughter, however, strategies to improve the prediction of such trait during animals' life has a potential to improve the farmers and industry decisions on strategies of commerce and genetic selection. Since meat tenderness had been recently correlated with oxidative stress, apoptosis, mitochondria and energetic metabolism [1,2], analysing blood metabolites might provide a clue of those aspects and facilitate the prediction of tenderness in animals live. In this line of reasoning, quality and impact of metabolomics studies depend on the sample preparation method. For that, this study has been designed to compare four sample preparation methods for beef cattle serum samples by assessing the spectral quality, metabolite identification, reproducibility, speed and relative cost.

II. MATERIALS AND METHODS

Blood samples (10 ml) were collected from five steers, samples were centrifuged for 15 min at 3000 × *g* at 4 °C; the serum was transferred into eppendorf tubes. Three technical replicates were used for each sample preparation method (a total of 12 samples). Samples were prepared following the methods: metabolites extraction using methanol and chloroform (M+C); methanol, chloroform and acetone (M+C+A); filtering the samples in a 3 KDa filter (3 KDa); filtering the samples in a 2 µM filter (2 µM) [3]. Metabolomics was evaluated through nuclear magnetic resonance spectroscopy (^{1}H-NMR). NMR spectra were recorded at 298 K using a 14.1 T Bruker spectrometer (600 MHz for hydrogen frequency), equipped with a 5 mm TCI cryoprobe. Spectra were processed using Chenomx Software v 10.0. Data were analyzed using MetaboAnalyst 5.0 (www.metaboanalyst.ca), Venn diagram was accessing the web tool Jvenn (http://jvenn.toulouse.inra.fr/app/usermanual.html). The methods were evaluated based on the spectra quality, reproducibility, easily processing, speed and relative cost [3].

III. RESULTS AND DISCUSSION

The use of 2 µM filters did not yield high-quality spectra for serum samples, leading to the exclusion of this treatment from further analysis. Principal Component Analysis (PCA) revealed a distinct separation between samples processed with the 3 KDa filter and those treated with chemicals, while an overlap was observed between samples treated with M+C+A and M+C. Twenty-nine metabolites were identified in serum samples processed using chemical methods, whereas a greater number (47 metabolites) was detected when the 3 KDa filter was employed. The filter also resulted in higher metabolite concentrations and improved reproducibility.

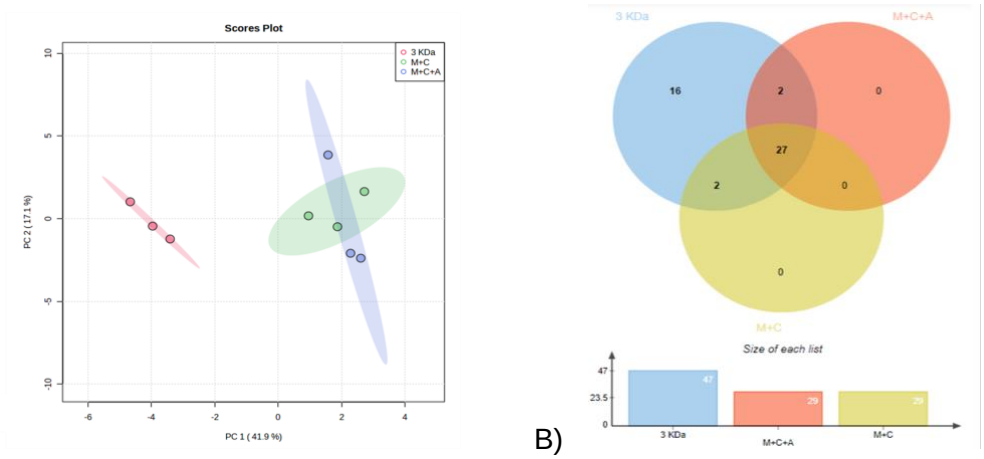

Figure 1. A) PCA scores plot of metabolites profile in serum according to different metabolites extraction methodology (red, 3 KDa; green, C+M; and dark blue, C+M+A). B) Venn diagrams of the number of extracted metabolites in cattle serum samples according to different metabolites extraction methodology (blue, 3 KDa; orange, M+C+A; and yellow, M+C).

Table 1 Qualitative scores of serum preparation according to different metabolites extraction methodology. Scores: 1 – poor; 2 – acceptable; 3 – excellent.

Method	Spectral quality	Metabolite's identification	Metabolite's quantification	Reproducibility	Speed	Relative cost	Total
M+C	3	2	2	2	1	3	13
M+C+A	3	2	2	2	1	3	13
2 µM	1	-	-	-	1	2	4
3 KDa	3	3	3	3	2	1	15

M+C: Methanol:chloroform (1:1, v/v); M+C+A: Methanol:chloroform:acetone (1:1:1, v/v/v); 3 KDa filters (Amicon Ultra -0.5, Merck Millipore Ltd. Brazil); and 2 µM filters (Analitica Scientific, São Paulo, Brazil). Adapted of Samuelsson et al. [4]

IV. CONCLUSION

The 2 µM filter is not suitable for removing macromolecules from serum samples. Conversely, the 3 KDa filter has been confirmed as a highly suitable method for preparing beef cattle serum samples for NMR-based metabolomics.

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