

Applying Solid-phase Extraction and Testing the Internal Standard used in Fatty Acid Analysis of Grass and Maize Silages

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Recent developments in fatty acid (FA) analysis of maize and grass silages were performed by application of direct transesterification (DT) method followed by purification by solid-phase extraction (SPE). Quantitative aspects of FA analysis by gas-liquid chromatography (GLC) were also studied. The application of the DT method to commercial silages containing ryegrass produced the highest amount of non-fatty acid methyl esters (non-FAME) compounds compared with maize silages. Three classes of non-FAME compounds were identified in silages containing ryegrass (phytadienes, organic acids and a sugar derivative product). In maize silages, the TCA (tricarballic acid, trimethyl ester), which is a derivative product of Fumonisin B, was also identified. The development of a SPE clean-up step significantly reduced the amount of non-FAME compounds in both silages. Five FAs (15:0, 17:0, 19:0, 20:0 and 21:0) were evaluated as internal standard (IS). The correction of the individual GLC areas using theoretical relative FID response factors or the correction of the natural content of the IS in the silages were also studied. The 15:0, 17:0 and 20:0 were naturally present in all silages samples, although their use as IS did not affect total FA composition. Corrections of GLC peak areas did not significantly influence total FA composition. The effect of the IS (without any GLC area correction) on the quantification of FA composition (mg/g DM) was only significant for some minor FAs. As conclusion, we suggest the use of the SPE step to reduce the amount of interfering compounds and we propose the 19:0 as IS, because it is not naturally present in samples, do not co-elute with other FAs in silages and elutes close to the majority of FAMES, minimizing the effects of different volatilities on the injection system.