Idea of rapid preparation of fatty acid methyl ester using *in situ* derivatization from fresh horse mussel†

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Abstract: The analysis of the fatty acid (FA) profile requires multiple preparation steps, which are lipid extraction followed by derivatization of the FA into fatty acid methyl ester (FAME). The procedures are time-consuming, generally require large volumes of sample sizes and solvents. This report proposes a technique for the preparation of FAME from fresh horse mussels without a step of lipid extraction. A rapid *in situ* derivatization using *N, N*-dimethylformamide dimethyl acetal (DMF-DMA) methylation followed by alkali-transesterification was examined. Direct alkali-transesterification of the fresh sample gave only 58.7% FAME with 12.4% triglyceride and 21.1% FFA. The alkali *in situ* method showed low conversion efficiency due to the initial sample contains high contents of moisture and FFA (75.11% of the fresh sample and 14.3% of total oil, respectively). The reaction was developed by using two steps in situ derivatization. A 50 mg sample was methylated with 1 mL of DMF-DMA (100 °C, 15 min), followed by transesterified with 10 mL of 1% (w/v) NaOH in methanol (60 °C, 3 min). The FAME yield of 79.9% with 7.8% triglyceride and 8.5% FFA was obtained. The two steps *in situ* derivatization gave a promising result with the higher conversion with lower FFA. However, increasing the conversion efficiency as well as the variety of samples should be further studied.

Keywords: Fatty acids; Fresh sample; *In situ* transesterification; Methylation; *N*, *N*-dimethylformamide dimethyl acetal