REPEATABILITY OF A CHLOROFORM SOXHLET EXTRACTION METHOD TO DETERMINE FAT CONTENT IN BEEF

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I. INTRODUCTION

Emerging technologies are being developed which will allow for the objective, precise and accurate measurement of intramuscular fat (IMF) in Australian beef and lamb and have potential to improve prediction of meat-eating quality for the local industry [1]. These technologies require a uniform gold-standard for IMF, enabling them to train upon and become accredited to predict this trait. Chloroform is a comparatively safe and effective solvent for fat extraction in beef meat samples [2], however there is limited evidence that its use in Soxhlet extraction is repeatable. Previous studies have shown Soxhlet fat extraction is improved with freeze drying and homogenisation [3] as it eliminates moisture fluctuations, minimises sample heterogeneity and increases surface area for contact with the solvent [4]. On this basis we hypothesised that Soxhlet fat extract of freeze dried and homogenised samples using chloroform solvent would be highly repeatable.

II. MATERIALS AND METHODS

Five beef reference samples were sourced from commercial suppliers (A to E), were shipped frozen to Murdoch University, Perth, Australia and stored at -20 °C until analysis [5]. Samples were thawed at 4 °C before being divided into sub-samples placed into pre-weighed tubes. The sub-samples were weighed prior to freeze drying and then re-weighed after drying to determine dry matter percentage (%) before being homogenised. Triplicate aliquots (3 g) were removed from each reference sample, placed into pre-weighed filter paper envelopes and then oven dried at 50 °C overnight. After drying, the samples were re-weighed to calculate the pre-extraction sample weight. All aliquots (n = 15) were inserted at the same time into the Soxhlet apparatus which was placed on a 3 L round bottomed flask containing chloroform (2.5 L), mounted on a heating mantle. The heater was set and adjusted to maintain a slow boil for a total extraction time of 72 hours. The samples were removed from the apparatus and any residual chloroform allowed to evaporate. The extracted samples were dried at 50 °C for three days before being stored in a bench top desiccator until weighing to calculate dry matter (DM%). This was repeated twice using the same protocol for a total of three extractions (45 aliquots in total). The fat content was calculated as the proportional difference between the pre- and post-extracted weight of the dried sample. This was then multiplied by DM% and reported on a wet matter basis (fat%) The fat% data was analysed using general linear models in SAS (SAS Version 9.1, SAS Institute) which included fixed effects for reference sample, triplicate and extraction.

III. RESULTS AND DISCUSSION

Aligning with the hypothesis, chloroform Soxhlet extraction demonstrated a very high level of repeatability across the broad range of fat content in the reference samples. There was a significant effect of reference standard ($P<0.01$, F value = 33,325.6) and extraction ($P<0.01$, F-Value = 13.6) and their interaction ($P<0.05$, F value = 2.99). This small effect was only observed for reference samples A, B and D (Table 1). The largest difference in fat content between extracts was found for sample A, where extract 1 was 0.7% higher than extract 2 ($P<0.001$, Table 1). Although the samples were freeze

dried and ground (both fresh and dried), some fluctuations in sample heterogeneity may have caused this small day of extraction effect either due to sample coarseness [6] and/or high fat content [7]. However, there was no effect of triplicate (P>0.05) within sample, further demonstrating the high degree of repeatability of the method.

Table 1 Predicted least squared means ± standard errors for fat content (%, wet matter basis) of beef determined by chloroform Soxhlet extraction.

Reference standard	Extract 1	Extract 2	Extract 3
A	35.9 ± 0.09[a]	35.2 ± 0.09[b]	35.6 ± 0.09[c]
B	28.7 ± 0.09[d]	28.2 ± 0.09[e]	28.4 ± 0.09[e]
C	20.6 ± 0.09	20.7 ± 0.09	20.7 ± 0.09
D	16.4 ± 0.09[f]	16.1 ± 0.09[g]	16.2 ± 0.09[fg]
E	12.2 ± 0.09	12.1 ± 0.09	12.1 ± 0.09

[a,b,c] Letters within rows that differ are significantly different (P<0.05)

IV. CONCLUSION

This study demonstrated that chloroform Soxhlet extraction was a precise and repeatable methodology across a wide range of fat content in beef samples, making this method suitable as an industry reference standard.

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