SHORT COMMUNICATION

Rapid determination of total CLA concentration in beef fat

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Aldai, N., Rolland, D. C., Kramer, J. K. G. and Dugan, M. E. R. 2007. **Rapid determination of total CLA concentration in beef fat**. Can. J. Anim. Sci. **87**: 181–184. Conjugated linoleic acid (CLA) has many potential healthful properties, and beef is naturally enriched with CLA. Simple and rapid methods to measure total CLA were investigated to enable sorting of beef carcasses with potential enhanced economic value. Direct alcohol extraction combined with measuring absorbance was simple, accurate and perhaps the most viable method for rapid carcass sorting compared to methods using saponification or methylation followed by extraction.

Key words: Beef, fat, conjugated linoleic acid, rapid methods

Aldai, N., Rolland, D. C., Kramer, J. K. G. et Dugan, M. E. R. 2007. **Dosage rapide de la concentration totale d'acide linoléique conjugué dans la graisse de bœuf**. Can. J. Anim. Sci. **87**: 181–184. L'acide linoléique conjugué (ALC) peut être bénéfique pour la santé à maints égards et le bœuf en possède naturellement une grande quantité. Les auteurs ont examiné des méthodes simples et rapides pour mesurer la concentration totale d'ALC afin de permettre le tri des carcasses susceptibles de rapporter davantage. L'extraction directe à l'alcool combinée à l'absorbance constitue une technique simple et précise, sans doute la plus viable pour identifier rapidement les carcasses. Elle est préférable aux techniques reposant sur la saponification ou la méthylation suivies d'extraction.

Mots clés: Bœuf, graisse, ALC, dosage rapide

Fats and oils are nutritionally valuable due to their high energy density and their ability to act as carriers for fat-soluble vitamins, and moreover, some of the fatty acids (FA) are essential because they cannot be synthesised by mammals (18:2n-6, 18:3n-3). The composition of dietary fats and oils in food affects human health (Ulbricht and Southgate 1991). Interest in conjugated linoleic acid (CLA) has increased considerably over the past decade due to its purported roles in prevention and possible treatment of several diseases including diabetes, obesity and some types of cancer (Belury 2002; Ip et al. 2003). Beef is naturally enriched with CLA as a by-product of biohydrogenation of PUFA by rumen bacteria. Levels of CLA in beef have, however, been found under some circumstances to be quite variable even when animals are consuming the same diet (Kelly et al. 1998). Differences in the rumen environment can alter the rumen microbial population and these determine the pathways and intermediates used during PUFA biohydrogenation (Demeyer and Doreau 1999; Lock and Bauman 2004).

Among the most complex and time-consuming methods used in FA analysis is the analysis of individual CLA isomers using a combination of techniques including gas chromatography (GC) with a 100 m polar column combined with silver ion high performance liquid chromatography (Ag^+-HPLC) (Cruz-Hernandez et al. 2004). This requires specialized training, ~4–5 h per sample (methylation, GC, HPLC, chromatogram integrations and data sumarization) and relatively expensive equipment, making it ill-suited for rapid sorting of carcasses or tissues with improved economic value due to their CLA content. The objective of the present study was, therefore, to determine if a rapid method for measuring total CLA concentration in beef fat could be developed using simple extraction techniques combined with absorbance measurements at 233 nm (specific for conjugated dienes).

Backfat samples used in this study were collected opportunistically over a period of 4 mo from 95 animals of mixed origin (i.e., from unrelated studies) slaughtered at the Lacombe Research Centre abattoir. All studies from which samples were collected were in compliance with Canadian Council on Animal Care (1993) guidelines. Samples first underwent preliminary GC analysis using a 30 m SP2340 column (Supelco, Bellefonte, PA) to determine the approximate CLA content. From the initial 95 samples, 24 were selected which were equally distributed across the full sample range (0.32 to 1.92% CLA in total fatty acids) and used

Abbreviations: **CLA**, conjugated linoleic acid; **FAME**, fatty acid methyl esters; **GC**, gas chromatography

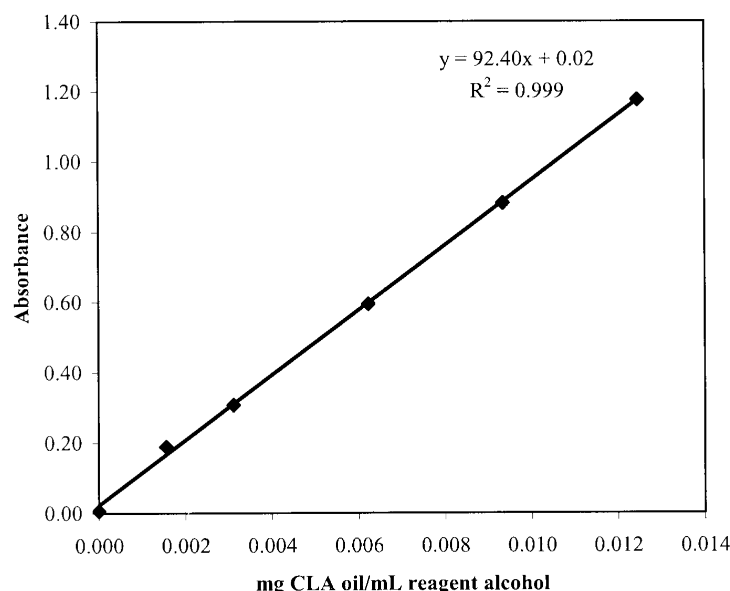

Fig. 1. Estimated linear regression parameters of CLA oil standard.

for extraction studies and for more thorough GC analysis (outlined below).

Reagent Alcohol Extraction

Extraction using reagent alcohol (90% ethanol, 5% methanol, 5% isopropanol) was investigated as a means to extract CLA as it has previously been demonstrated to be highly efficient at extracting lipids from selected tissues (Lucas and Ridout 1970). Fifty milligrams of backfat were homogenized for 1 min in 5 mL reagent alcohol. Samples were centrifuged (1000 × *g* for 5 min) and an aliquot of the supernatant was diluted to 0.5 mg mL^{-1} with reagent alcohol prior to reading absorbance at 233 nm.

Direct Saponification

Fifty milligrams of backfat was saponified in 5 mL 0.3 N methanolic potassium hydroxide at 80°C for 1 h (Cyberlipid Centre 2004). The non-saponifiable fraction was then removed by extracting the aqueous phase three times with hexane. The saponifiable fraction was then neutralized with 300 µL of 6 N hydrochloric acid and then extraced three times with hexane. The saponified extract was dried under nitrogen, dissolved in reagent alcohol, and diluted to 0.5 mg mL^{-1} with reagent alcohol prior to reading absorbance at 233 nm.

Direct Methylation

Fifty milligrams of backfat with 5 mg of C19:0ME as internal standard was dissolved in 0.5 mL benzene and methylated using 2 mL 0.5N sodium methoxide at 50°C for 15 min (Yurawecz et al. 1999). The FA methyl esters (FAME) were then extracted with hexane, purified using Supelclean LC-Si SPE cartridges (Supelco, Bellefonte, PA), dried under nitrogen, and dissolved in hexane. An aliquot of FAME extract was dried and resuspended in reagent alcohol to 0.5 mg mL^{-1} prior to measuring absorbance at 233 nm, and another aliquot of FAME extract was diluted to 1 mg mL^{-1} and analyzed using GC procedures described by Cruz-Hernandez et al. (2004) for the thorough analysis of CLA composition. Specifically, a Varian 3800 GC (Varian, Walnut Creek, CA) was used, equipped with a flame ionization detector, an automatic sample injector, and a Chrompak CP Sil 88 column for FAME analysis (100 m × 0.25 mm i.d., 0.2 µm film thickness; Varian - Chrompack, Middelburg, the Netherlands). Hydrogen was used as the carrier gas with a column head pressure of 25 psi (and a flow rate of approximately 2 mL min^{-1} measured at 45°C). Operating conditions included the following: initial temperature of 45°C and held for 4 min; programmed at 13°C min^{-1} to 175°C and held for 27 min; programmed at 4°C min^{-1} to 215°C and held for 35 min. Injector and detector temperatures were both held at 250°C. Chromatograms were integrated using Varian Star Chromatography Workstation 6.01 Software (Varian, Walnut Creek, CA).

Conjugated linoleic acid methyl esters were identified based on peak retention times using a GC reference standard with four positional CLA isomers (mixture #UC-59M; Nu-Chek-Prep Inc.; Elysian, MN), and quantified using peak area and C19:0 FAME as an internal standard.

Absorbance Measurements

A standard curve was developed using CLA oil (65% total CLA with equal amounts of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers). The CLA oil was diluted in reagent alcohol over a linear range (0.002 to 0.0125 mg CLA mL^{-1} reagent alcohol), which encompassed the range of sample concentrations in the present study. Sample absorbance was measured at 233 nm using a Pharmacia Biotech UltroSpec 3000 (Cambridge, UK) and CLA concentrations were determined

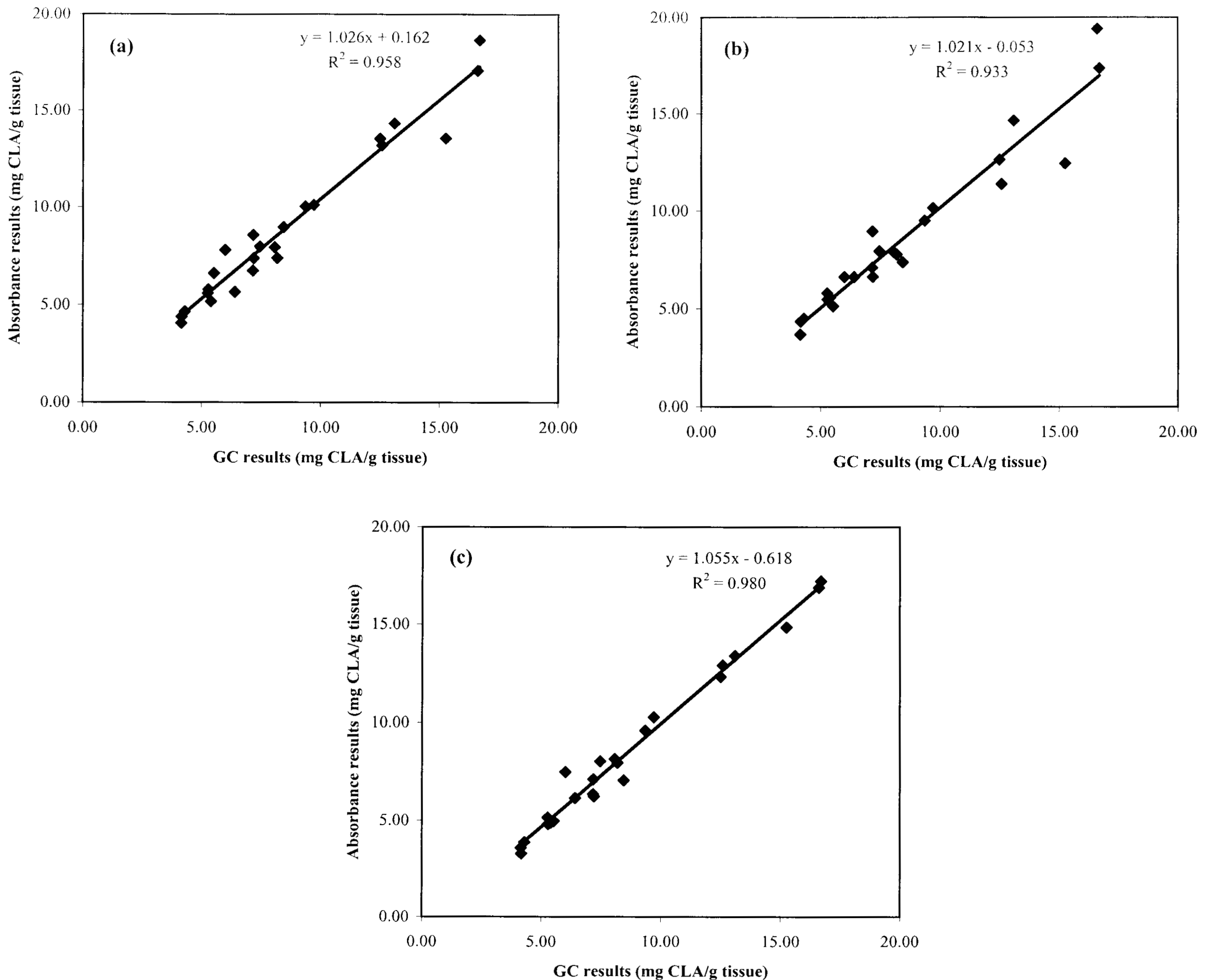

Fig. 2. Linear regression estimated between reagent alcohol extraction (a), direct saponification (b) and direct methylation (c) – absorbance measurements and comprehensive total CLA determination (100 m column, Cruz-Hernandez et al. 2004).

using the molar absorption coefficient calculated from the standard curve.

Statistical analyses included regressions of extraction and GC analyses and a one-way ANOVA comparing actual and predicted CLA concentrations from regression analysis for the three extraction procedures using the GLM procedures of SAS (SAS Institute, Inc. 2001).

A typical standard curve regressing absorbance at 233 nm and CLA concentration is presented in Fig. 1 ($R^2 = 0.999$) indicating near perfect linearity between absorbance and the CLA concentrations across the standard range. Regressing CLA concentrations determined by GC analysis with those obtained by reagent alcohol (a), saponification (b) and methylation extraction (c) methods resulted in R^2 values of 0.96, 0.93 and 0.98, respectively, and significant linear relationships for all methods ($P < 0.001$; Fig. 2).

A comparison of the GC results with those obtained using the three different extraction methods showed that saponification with methanolic potassium hydroxide prior to extraction tended to yield more variable results than those obtained with the other two methods (direct extraction and direct methylation) ($P < 0.10$). On the other hand, direct methylation with sodium methoxide in methanol showed the highest R^2 value, but this method was far more time-consuming than direct extraction using reagent alcohol. Moreover, a base-catalyzed transesterification method is not suitable for esterifing free FA (as reviewed in Aldai et al. 2005), which may lead to an underestimation in CLA content. In general, however, tissue free FA levels are low unless samples have not been kept cold enough during processing and storage (Kramer and Hulan 1978). Direct extraction using reagent alcohol followed by measuring

absorbance at 233 nm is by far the simplest and most viable method for the rapid sorting of beef carcasses with differing CLA contents. The accuracy of the direct extraction procedure for total CLA determination was shown to be acceptable compared with the more reliable GC method, and is certainly more practical under industrial conditions. The speed and accuracy of this method would, however, need to be tested under high capacity industry conditions. It should also be emphasized that the rapid methods described here represent the sum of all CLA isomers that absorb at 233 nm, making no attempt to determine the individual CLA isomers. The latter requires more elaborate analytical techniques described elsewhere (Cruz-Hernandez et al. 2004).

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