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Prediction of naturally-occurring, industrially-induced and total *trans* fatty acids in butter, dairy spreads and Cheddar cheese using vibrational spectroscopy and multivariate data analysis

Ming Zhao [a, b], Renwick J. Beattie [c], Anna M. Fearon [c], Colm P. O'Donnell [b], Gerard Downey [a, b, *]

[a] Teagasc Food Research Centre Ashtown, Dublin 15, Ireland
[b] School of Biosystems Engineering, University College Dublin, Belfield, Dublin 4, Ireland
[c] Agri-Food and Biosciences Institute, Newforge Lane, Belfast, UK

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ABSTRACT

This study investigated the use of vibrational spectroscopy [near infrared (NIR), Fourier-transform mid-infrared (FT-MIR), Raman] and multivariate data analysis for (1) quantifying total *trans* fatty acids (TT), and (2) separately predicting naturally-occurring (NT; i.e., C16:1 t9; C18:1 *trans*-n, n = 6 … 9, 10, 11; C18:2 *trans*) and industrially-induced *trans* fatty acids (IT = TT − NT) in Irish dairy products, i.e., butter (n = 60), Cheddar cheese (n = 44), and dairy spreads (n = 54). Partial least squares regression models for predicting NT, IT and TT in each type of dairy product were developed using FT-MIR, NIR and Raman spectral data. Models based on NIR, FT-MIR and Raman spectra were used for the prediction of NT and TT content in butter; best prediction performance achieved a coefficient of determination in validation (R^2V) ~ 0.91–0.95, root mean square error of prediction (RMSEP) ~ 0.07–0.30 for NT; R^2V ~ 0.92–0.95, RMSEP ~ 0.23–0.29 for TT.

1. Introduction

In Western Europe, annual dairy product consumption contributes up to 30% of individual intake of animal fat (FAO, 2013). Fat in dairy products originates from raw bovine milk, which contains about 3.5% milk fat; it typically consists of saturated fatty acids (SFA; 70%, w/w), monounsaturated fatty acids (MUFA; 25%, w/w) and polyunsaturated fatty acids (PUFA; 5%, w/w) (Grummer, 1991). Unsaturated fatty acids consist of *cis*/*trans* double bond isomers and conjugated double bond isomers. *Trans* fatty acids (TFAs) have been defined as "all the geometrical isomers of monounsaturated and polyunsaturated fatty acids having non-conjugated, interrupted by at least one methylene group, carbon–carbon double bonds in the *trans* configuration" (Codex Alimentarius, 2006). *Trans* PUFA "have at least one *trans* double bond and may therefore also have double bonds in the *cis* configuration" (EFSA, 2010). *Trans* fatty acids with conjugated double bonds (not separated by a methylene group) are excluded by these definitions (FDA, 2003), and hence, conjugated linoleic acid (CLA) isomers which occur naturally in bovine milk are not included in the TFA category as defined by these agencies.

Trans fatty acids in foods arise either as naturally-occurring or industrially-induced compounds. Industrially-induced TFAs (IT) are mainly formed during the partial hydrogenation or deodorisation of oils or fats during refining or cooking (Kodali, 2005). Naturally-occurring TFAs in dairy products include *trans* isomers of C18:1, C18:2, C18:3 and *trans*-palmitoleic acid (C16:1 t9), a biomarker of dairy consumption. These TFAs arise in the rumen of ruminants during extensive hydrolysis and bio-hydrogenation of unsaturated fatty acids contained in feed by the rumen bacteria (IDF, 2013). Industrially-induced TFAs present in dairy products have generally been added in the form of partially hydrogenated oils which have been included to manipulate the physical properties of dairy products (Fearon, 2011; Wright, Scanlon, Hartel, & Marangoni, 2001).

Some controlled dietary intervention studies indicate that TFAs from industrial and natural sources have different effects on cardiovascular risk factors in humans; a high dietary intake of TFAs from natural sources was found to have a neutral effect on plasma lipid and other cardiovascular disease risk factors whereas intakes

* Corresponding author. Tel.: +353 1 8059500.
E-mail address: gerard.downey@teagasc.ie (G. Downey).

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of industrially-induced TFAs seem to have a specific high density lipoprotein cholesterol-lowering property (Chardigny et al., 2008; Motard-Bélanger et al., 2008). Based on these observations, quantification of total TFAs and separate identification of naturally-occurring TFAs has become an important research task to assist in meeting both labelling and consumer information requirements.

The most accurate and common method used for TFA identification and quantification is gas-chromatography (GC) analysis. This method requires extraction of fat from the food matrix and preparation of the fatty acids as volatile methyl esters, thus destroying the sample and requiring significant time and resources. Therefore, vibrational spectroscopic techniques (NIR, MIR and Raman) have been explored to provide non-destructive and rapid measurements. Vibrational spectroscopy is based on quantum energy shifts when incident photons excite specific chemical bonds in sample material under reflection/transmission (IR) and anti-stokes/stokes scattering (Raman) modes. Since the 1940s, IR spectroscopy has been used to determine *trans* content in edible fats and oils (AOCS, 1946; Dutton, 1974; Firestone & De La Luz Villadelmar, 1965; Sleeter & Matlock, 1989). The American Oil Chemists' Society have published official IR methods (AOCS, 1999, 2000, 2009) but these methods are only suitable for edible oils and fats which are typically composed of a relatively small number of different fatty acids (approximately several dozen) compared to the over 400 in milk fat; they haven't achieved accurate prediction at low concentrations (<5% w/w of total fat) in complex matrices such as milk fat (Mossoba et al., 2011; Stefanov et al., 2011). Dispersive MIR spectroscopy has previously been investigated for the prediction of the fatty acid composition of bovine milk (Soyeurt et al., 2006, 2011). NIR was demonstrated for the same purpose in bovine milk (Coppa et al., 2010) and in some specific cheese varieties (Lucas, Andueza, Ferlay, & Martin, 2008). Recently the use of MIR and NIR instruments for the prediction of fatty acids in fresh and thawed bovine milk has been reported (Coppa et al., 2014). FT-MIR has also been used to determine fatty acid composition of butter and margarines (Safar, Bertrand, Robert, Devaux, & Genot, 1994), and FT-Raman has been reported to detect TFAs in a study limited to raw milk samples only (Stefanov et al., 2011). No published reports have described the investigation of spectroscopic methods to quantify naturally-occurring and industrially-induced TFAs in dairy products.

The objectives of this study were to investigate the use of vibrational spectroscopy (NIR, FT-MIR and Raman) and multivariate data analysis for, (1) quantifying TT, and (2) separately predicting NT and IT in Irish butter, dairy spreads and Cheddar cheese.

2. Materials and methods

2.1. Samples

In total, 60 butter, 54 dairy spreads and 41 Cheddar cheese samples were collected at regular intervals over the course of one year (4 July 2012 to 5 July 2013). Samples of butter and Cheddar cheese were supplied by a well-known Irish manufacturer; dairy spread samples from four major Irish brand names: Dairygold, Kerrymaid and Lowlow (Kerry Foods Ltd., Cahirciveen, Ireland), Flora (Unilever, Kilkenny, Ireland), were purchased at approximately two-week intervals in different retail outlets in the Dublin city area. Fresh samples were stored at −4 °C for less than two days before subsampling into screw cap containers (butters and dairy spreads) or aluminium foil (cheese) prior to storage at −20 °C.

2.2. Determination of trans fatty acids by GC

2.2.1. Fat extraction

Butter and dairy spreads are mainly fat-based whereas cheese has a much higher protein content, therefore different methods of fatty acid extraction were carried out on these two sample groups. The fat and non-fat portions of the butters and dairy spreads were separated by first melting in an oven at 40 °C and then centrifuging at 1591 × *g* and 15 °C for 10 min (Versatile centrifuge, Sigma 2–16 PK; Sigma, Osterode am Harz, Germany). The top fat layer was then removed and methylated as below. Cheese samples were grated, mixed with ammonium thiocyanate (30%, w/v) and then shaken in a thermal shaker at 60 °C for 1 h at 135 rpm before centrifugation at 561 × *g* and 30 °C for 20 min. The upper fat layer was removed and methylated as below.

2.2.2. Methylation

Extracted fat samples were melted at 60 °C and an aliquot (40 μL) of each sample transferred into a glass tube; 5 mL *n*-hexane and 2 M KOH solution (200 μL) were then added and mixed using a vortex mixer for 40 s. Tubes were left on the bench at ambient temperature for 5 min after which sodium hydrogen sulphate monohydrate (0.5 g) was added to each tube before centrifugation at 223 × *g* and 15 °C for 5 min. Afterwards, 120 μL of the methylated sample and 780 μL of *n*-hexane were transferred into a 2 mL vial prior to GC analysis. All chemicals used were from Sigma.

2.2.3. GC analysis

Methylated samples were analysed following injection onto a gas–liquid chromatograph (Varian 3400 Capillary GC; Varian, Walnut Creek, CA, USA) fitted with a flame-ionisation detector and a CP Sil 88 capillary column (100 m × 0.25 mm × 0.20 μm; Chrompack, Middleburgh, The Netherlands). Separation of fatty acid methyl esters (FAMEs) was carried out as previously described (Coakley et al., 2007). Each chromatograph comprised peaks in a plot of retention time (min) versus voltage (volts) from which the area under each peak was expressed as a percentage of FAME detected. Data collection and integration were carried out using Varian Star Chromatography Workstation (version 6.0; Varian Inc., Walnut Creek, CA, USA) and Compass CDS (version 2.0; Bruker, Amundsenweg, The Netherlands).

To ensure comparability of columns and accuracy of TFA results, reference materials were developed at Teagasc (Moorepark, Ireland) and regularly assessed during the analysis. TFAs were detected in the dairy products tested as follows: C14:1 t9, C15:1 t10, C16:1 t9, C17:1 t10, C18:1 t6-t9, C19:1 t7, C19:1 t10, C18:2 t9 t12, C18:2 t,c; c,t, C20:1 t11 and C22:1 t13. TFA standards were purchased in methylated form (Nu-chekprep Inc.; Elysian, MN, USA). Analysis of each TFA was carried out three times on separate occasions to determine the average retention time of each TFA. Polyunsaturated TFAs (C18:2 t,c; c,t) were detected between the retention times of C18:2 t, t and C18:2 c,c (linoleic acid) on the chromatograph. All the peaks in this retention time range were taken to be a C18:2 t,c; c,t group. The entire peak area of C18:1 t6 - t11 was categorised as the C18:1 *trans* group because further temperature programming was able to show overlapping between *trans*-n; *trans*-n and *cis*-n of C18:1 isomers. Samples were analysed in random order; one out of every three or four samples was analysed in duplicate to determine the consistency of the results.

Naturally-occurring TFAs (NT) include the C18:1 *trans* group (C18:1 *trans*-n n = 6 … 9, 10, 11), C18:2 *trans* group and C16:1 t9. Trace amounts of naturally-occurring TFAs in the C18:1 *trans* group (n = 12, 13 … 16) and C18:3 *trans* group have not been included in this study due to the separation limits and detection thresholds of the GC analysis used. All fatty acids detected in this work, which are